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VOLUME 8

J. BĚLEHRÁDEK, TEMPERATURE AND LIVING MATTER

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VOLUME 8

TEMPERATURE AND LIVING MATTER

BY

J. BĚLEHRÁDEK

PROFESSOR OF GENERAL BIOLOGY, CAROLINE UNIVERSITY, PRAGUE

WITH 70 ILLUSTRATIONS

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AND
LIVING MATTER

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BY

J. BĚLEHRÁDEK

PROFESSOR OF GENERAL BIOLOGY, CAROLINE UNIVERSITY, PRAGUE

With 70 Illustrations

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PREFACE

"Biology, like theology, has its dogmas. Leaders have their disciples and blind followers."

H. F. Osborn

"Le progrès des sciences rend inutiles les ouvrages qui ont le plus aidé à ce progrès."

Anatole France

The production of papers on the biological action of temperature has increased to such an extent in the last ten years that monographs of earlier date can no longer be considered reliable. The present book is an attempt at a concise, but well documented summary of the facts and theories hitherto published. Thus it brings up to date the results collected by Kanitz and Przibram in their excellent monographs published in 1915 and 1923 respectively, but it also includes a more detailed discussion of certain problems which have not lately been treated in a more general and comparative way, notable among them being the action of temperature upon the physical, biochemical and morphological properties of living systems, the action of frost and of low temperatures above zero, various aspects of the action of heat, the stimulatory effects of temperature variations, and the mechanism of thermal adjustment. Likewise in sections dealing with the temperature coefficients of vital functions many facts and views of more recent origin have been discussed. In general the nature of the problem made it necessary to deal not only with facts, but with theories as well, the latter being discussed perhaps at greater length than is customary in other branches of biology.

The general lines laid down for this series necessitated the limitation of subject-matter to the cytological and physico-chemical aspect of the action of temperature upon living systems. The question of the response to temperature of the organism as a whole has been therefore largely omitted, but a few of the more recent attempts at a physico-chemical interpretation have been discussed. Even after the subject of the monograph has been thus limited, the literature now at hand is so vast that it was found necessary to give up all idea of a complete bibliography. All important papers appearing up to the end of the year 1933 have been taken into account, and numerous papers and books are quoted, which themselves contain exhaustive bibliographies on special subjects.

Thanks are due to Professor Friedl Weber, Graz, Austria, to Mr. Stuart E. Mann, Lecturer in English at the Masaryk University, Brno, Czechoslovakia, for his careful linguistic arrangement of the text; to Messrs. Borntraeger, the publishers of these Monographs, who made no restrictions as to the scope of the book, and thus went far beyond the limit usually allotted to such a work as this.

The author is further indebted to the Czechoslovak Ministry of Public Instruction for a subsidy which enabled him to procure certain works which otherwise could not have been obtained in their original form.

Brno, June 29, 1934

Jan Bělehrádek

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CHAPTER I

GENERAL PRINCIPLES OF BIOLOGICAL TEMPERATURE ACTION

1. Limits of biokinetic temperatures

Temperatures at which life processes normally occur and which we shall call, as proposed by KANITZ (1925), biokinetic temperatures, occupy a narrow zone at the lower end of the temperature scale (fig. 1). Even in those organisms which are specially adapted to extreme temperatures, the limits are still relatively narrow (—273—+150°).

The question arises why this should be so.

A more detailed inquiry into the relations between temperature and integration of matter enables us to gain some insight into the problem. The general temperature scale, limited at its lower end by absolute zero, has also a theoretical upper limit which has been recently calculated by POKROWSKI (1928) in two different ways thus:

$$T_{max} = 3.10^{12} \qquad \text{(I)}$$

that is three billion degrees. At this temperature the density of energy in particles of matter would become equal to the density of energy in the surrounding space, which practically means that matter necessarily would cease to exist as such, *i. e.* in the form of material particles. At a somewhat lower temperature matter can exist only in the form of free electrons and atomic

Fig. 1. Lower end of the absolute temperature scale, up to 4000°.
C = melting point of carbon, *Pt* = m. p. of platinum, *Al* = m. p. of aluminium. — Black space: biokinetic temperature zone. Dotted space: temperature interval supported by dry protoplasm.

nuclei. On further cooling free atoms and ions are constituted. Eddington (1927) estimates that at a temperature of about 40,000,000°, which he calculates to exist in the interior of the sun, free atoms are present together with free electrons and with mutilated atomic nuclei. At lower temperatures atoms may combine into molecules, and when the temperature descends to a still lower level, *i. e.* when it approaches absolute zero, large molecules and colloidal particles may be formed.

The nature of matter in general is to form more and more complex entities as the temperature falls. Living matter is no exception to this rule. If it may be supposed that the general structural forces are the same in the inorganic and organic world (W. J. Schmidt 1924), it may be said that life is restricted to the lower end of the temperature scale because of its high chemical complexity.

2. General outline of temperature action upon living systems

Although there are many important differences among organisms and in different parts of one and the same organism as to the range of biokinetic temperatures, sensitivity to high and low temperatures and to the alterations induced by temperature variations, a general scheme of biological temperature action may be roughly outlined.

(*a*) Rate of biological processes generally increases on heating and decreases on cooling within the limits of the biokinetic temperatures. The degree of this change is described in terms of temperature coefficients.

(*b*) Temperature variations within the biokinetic zone produce changes in chemical, physical and morphological properties of organisms and of their parts.

(*c*) When the lower limit of the biokinetic range is reached, biological reactions are arrested. So long as the exposure is not too long, the process is reversed on heating. This phenomenon, generally called chilling (chill coma), may occur at temperatures above or below 0° C, according to the specific sensitiveness of the system.

(*d*) When the temperature of a living system decreases below its freezing point, the system freezes and ice is formed. This

phenomenon is in many instances preceded by a state of supercooling. When ice is once formed, the system may or may not recover on subsequent heating.

(*e*) When the upper biokinetic limit ("optimum") is passed, the velocity of biological reactions decreases to a complete stop, a phenomenon which is generally reversed on cooling. On further heating or at longer exposures an irreversible disintegration of protoplasm takes place.

(*f*) When temperature is suddenly raised or lowered within the biokinetic limits or slightly outside them, living systems are stimulated.

(*g*) There is a great variation in the susceptibility of different organisms, and in the extent to which they change according to principles enumerated in *(a)—(f)*. Age, adaptation and other internal factors, as well as many external factors, are the causes of this variation.

(*h*) Many organisms tend to become more or less completely independent of external temperature variation by virtue of various regulating mechanisms, namely heat production, heat economy, heat liberation, evaporation, etc.

In this monograph, only the topics enumerated under *(a)—(g)* are discussed. Regulating processes are largely omitted here because of their rather remote relation to the biology of living matter.

3. Rate of temperature changes within living systems

When a body A whose temperature is T_o is put into a medium whose constant temperature is T_n, the temperature equilibrium will be established in time according to the law of Newton:

$$\vartheta = a \cdot e^{-bt} \qquad \text{(II)}$$

where ϑ is the difference of temperatures reached in the time t, a and b being constants. P. JENSEN

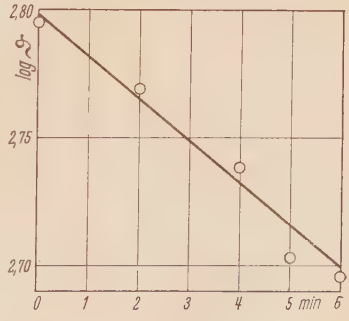


Fig. 2. NEWTON's law tested on muscle (JENSEN 1913). Abscissa: time of exposure. Ordinate: *log* of temperature difference between the muscle and the bath.

(1913) tested the rule on muscle tissue and found that the law holds good down to the freezing point of muscle. When the law holds good, the logarithm of ϑ plotted against time gives a straight line. Fig. 2 is a graphical representation of an experiment in which the temperature of a muscle cooled gradually in a bath, was measured thermoelectrically.

In general the thermal conductivity of living matter is low. In watery tissues it approaches that of water, yet it may decrease to one third of this value in fatty tissue (Breuer 1924).

Table I

Substance	*Thermal conductivity* (cal/cm sec. ^{0} C)
Copper	0.9
Water	0.0014
Muscle	0.001
Fatty tissue	0.0003
Wood	0.0001—0.0008
Wool	0.00009

Structurally polarised bodies, *e. g.* crystals, show different values in different planes. As far as thermal conductivity of living matter has been studied, no such differences can be detected (in muscle, Breuer 1924).

4. External and internal temperature

Owing to their low thermic conductivity, living systems establish temperature equilibria relatively slowly when placed at a new constant temperature. It is a common practice in biological work to consider the internal temperature of the (poikilotherm) organism under investigation as identical with the surrounding temperature, when a definite time has been allowed for the equilibrum. Yet this is not always so, and important differences between internal and external temperatures may arise when the organism is heated by absorption of light or any other form of radiation, or by its physiological activity, or when it is cooled by evaporation.

Living organisms are heated by incident rays when the amount of heat into which the radiant energy absorbed per unit of time is finally transformed is great compared with the amount

of heat which can escape from the body. Various pigments may increase the heat effect of radiations, as for instance the red pigments in plants. A. M. Smith (1907) reported that leaves with red pigments are, at a given external temperature, 2—4° warmer than analogous leaves without such pigments (compare further Fritzsche 1933, Seybold and Brambring 1933). Sonne (1920) estimates that in human skin the temperature may attain 48° during exposure to sunlight owing to the high absorption power of haemoglobin.

As to the excess of body temperature over the external temperature in poikilotherms during muscular and other activity, numerous examples are found in Lefèvre's (1911) monograph. The excess may amount to one or more degrees not only in large animals, but also in small ones. (See also Semper 1880, Tigerstedt 1910—1914.)

On the other hand, organisms may cool themselves by evaporation. This is a common phenomenon in plants, but has been reported as well for animals. (See especially for insects: Necheles 1924, Buxton 1924, 1931, Sayle 1928 *a*, *b*; for reptiles and amphibians: Hall and Root 1930.) Necheles finds that in the cockroach the body temperature follows external temperature variations only between 13—23°, while at temperatures above and below this range regulating processes partly counterbalance the external effects (see further Gunn 1935, Kozhantschikov 1933).

5. Specific heat of living matter

In animal calorimetry the specific heat (number of calories necessary to raise the temperature of 1 g of a body 1° C) of living bodies is generally estimated as equal to 0.80—0.83 (Mareš 1892, C. J. Martin 1903). Rosenthal (1878) obtained the following figures by direct measurements:

Table II

Substance	Specific heat
Blood (defibrinated)	0.927
Striated muscle	0.825
Fatty tissue	0.712
Bone, compact	0.300
Bone, spongy	0.710

The same author showed that in dried muscle the value drops to 0.330 and suggested that the specific heat of tissues is related to their water content. Yet according to more recent investigations of Balakirew (1929) such generalization is not admissible, because the specific heat of a living system is not an average of specific heats of its chemical constituents, but depends also on the physical state of the system.

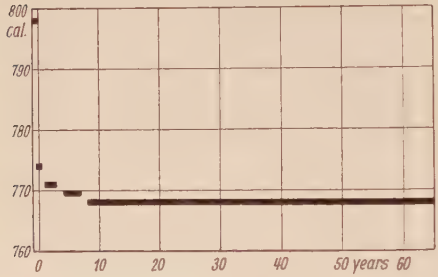


Fig. 3. Specific heat (ordinate) of human brain tissue in function with age (abscissa) (Balakirew 1929).

Balakirew demonstrated further that the specific heat of the central nervous system varies with the animal species, with the region of the organ, with age, and with the time elapsed since death. In man the values vary with age as follows (see also fig. 3):

Table III

Age	Specific heat of brain tissue
Foetus (8 months)	0.798
Newborn	0.774
1—3 years	0.772
4—7 „	0.769
8—65 „	0.768

CHAPTER II

RATE OF BIOLOGICAL PROCESSES AT BIOKINETIC TEMPERATURES

1. General remarks

It has long been known that temperature affects the rate of processes in living organisms to a relatively high degree. The first attempts of biologists at a mathematical representation of this phenomenon may be traced back far into the past, long before chemists proposed to apply to living nature those chemical laws which govern the rate of processes in the inanimate world. The purpose of such attempts is to compare living systems with each other or with inanimate systems in the hope of gaining material for theoretical speculation as to the mechanism and nature of biological processes. This plan involves a double task, *i. e.* a study of the temperature coefficients of biological processes, and their theoretical explanation. In the present chapter the various temperature formulae applied in biology will be discussed with a view to obtain temperature coefficients for a legitimate comparison. The theoretical views which have been developed of late with regard to the meaning of temperature coefficients in biology, will be discussed in Chapter IV.

When dealing with temperature formulae in biology it is necessary to keep in mind that biological phenomena often are of such a complex character that equations which have proved correct in physics and chemistry, do not fit in closely enough with biological experimental data. Furthermore it cannot be expected that a single temperature formula could embrace all the variety of biological processes (Bělehrádek 1930*a*, D. E. S. Brown 1930*a*). Many secondary factors which themselves vary with temperature may disturb the primary effect of temperature (solubility of gases and of other substances; formation of additive

compounds at lower temperatures; crystallisation; etc.) so that in some cases the effect of temperature appears to be so irregular that there is no possibility at all of applying any simple equation (Piéron 1922, Mangold 1926, Ponder and Yeager 1930). These difficulties are increased further in cases where regulative, compensatory and adaptive processes cannot safely be excluded.

All temperature formulae in biology therefore are of a merely descriptive value and their use even in this restricted sense is necessarily limited by the above mentioned obstacles. All the more caution is necessary, therefore, in explaining the theoretical basis of temperature coefficients in biology (Chapter IV). In spite of the numerous temperature formulae used hitherto in biology there is none which can be said to hold good within reasonable limits in every case, neither is there a rational temperature formula in biology. Even those formulae which are rational in chemistry, are merely empirical in biology (see Bělehrádek 1930a). In the following paragraphs and chapters the term "temperature coefficient" will be used generally to denote any constant which means the degree of acceleration when temperature is raised a definite number of degrees. In special cases however use will be made of particular temperature coefficients which will always be denoted by their usual symbols (Q_{10}, μ, b, etc.).

2. Rule of thermal summation

The earliest attempt at a mathematical treatment of the relation between the temperature and the rate of biological processes was made by Réaumur, two centuries ago. Réaumur (1735) showed that the moment of ripening of fruits depends on the sum of average daily temperatures during the vegetation period. Also P. Adanson (quoted in A. P. de Candolle 1833) supposed that the moment at which plant buds develop into leaves may be calculated on the same basis. The idea of thermal summation, which is represented by the following formula:

$$y \cdot t = K, \qquad \text{(III)}$$

in which y = time, t = temperature, K = constant, was developed further chiefly by Boussingault (1844, 1851) and by Burckhardt (1860), while A. P. de Candolle (1833—1835), A. de Candolle (1855, 1865, 1875) and Sachs (1860*a*) criticized its use.

Some botanists suggested that the rule fits closer to the bservations when temperature is reckoned from the minimum emperature instead of from 0° C. If the minimum or threshold emperature (α) is introduced into the estimate, the formula ecomes:

$$y(t-\alpha) = K \qquad \text{(IV)}$$

his correction was used by Burckhardt (1860), A. de Can-olle (1865), V. Oettingen (1879) and by other botanists.

Also in entomology the rule of thermal summation has ound and still is finding a wide application, the threshold emperature being usually taken into account (see especially: Jennings 1907, Sanderson 1908, 1910, Pierce 1916, Blunck 924, Bodine 1925, Bodenheimer 1927, 1928, 1930, Bodenheimer nd Klein 1930, Shelford 1927, 1929, 1930, Heller 1928, udwig 1928*b*). In modern botany the formula is not used very ften (Stevens 1917*b*, Figurovsky 1930).

Some authors have tried with some success to apply the ule also to the rate of development in fishes (Reibisch 1902, Haempel 1913).

. Linear relation between temperature and velocity

Many authors have claimed that the relation between tem-erature and the velocity of biological processes is linear. . N. Harvey (1911) found this o be the case in the nervous onduction of the medusa *Cassiopea xamachana*, Knowlton and tarling (1912) in heart fre-uency in mammals, Ganter 1912) in nervous conduction in he frog, and A. G. Mayer (1914) n pulsation frequency of the nedusa *Aurelia aurita* (fig. 4). rogh (1914*a*, *b*) laid special tress on the possibility that at east in some biological processes he rate increases proportionally ith temperature, and gave some xamples of this. More recently has been found by different investigators that the same relation olds good in various vital phenomena (heart: Hsü and

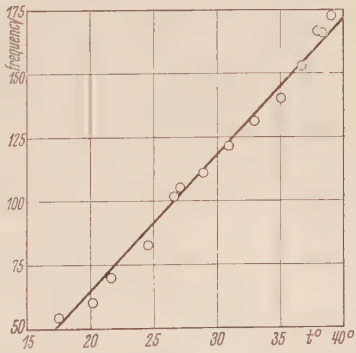


Fig. 4. Linear relation between temperature (abscissa) and pulsation frequency (ordinate) in a medusa (A. G. Mayer 1914).

Walbaum 1923, Barcroft and Izquierdo 1931, N. B. Taylor 1931; photosynthesis: Yabusoe 1924; growth in Ankylostomum: McCoy 1930; respiration in the bee: Betts 1929*a*; oxygen consumption in homoiotherm tissue: Schmutzler 1930; locomotion in the leech: Herter 1928). A general discussion of the formula is to be found in: Snyder (1913*a*, *b*), Kanitz (1915), Bayliss (1918), Janisch (1925, 1930), Pütter (1927), Bělehrádek (1929, 1930*a*).

In its mathematical form the relation may be written:

$$v = k \cdot t \qquad \text{(V)}$$

v being the velocity, t the temperature and k the constant.

It is evident that the rule of thermal summation, analysed in the preceding section, and the linear relation between velocity and temperature may be reduced to a single expression, in as much as velocity (v) is the reciprocal of time (y):

$$v = \frac{1}{y} \qquad \text{(VI)}$$

If we put

$$k = \frac{1}{K}, \qquad \text{(VII)}$$

the formula becomes:

$$yt = K \qquad \text{(VIII)}$$

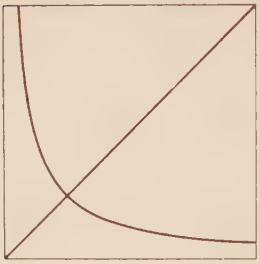


Fig. 5. Rectangular hyperbola and her reciprocal, straight line. Abscissa = temperature. When time is plotted as ordinate, the result is a hyperbola. When the ordinate is velocity, the straight line results.

This equation is identical with that expressing the rule of thermal sums (III). In graphic representation this rule gives a rectangular hyperbola, which is the reciprocal curve of the straight line, assumed by the rule of linear relation (fig. 5).

4. Q_{10} rule

The rule of Q_{10}, commonly used in biology, may be derived from a formula which was proposed by Berthelot (1862) as a first approximation for the rate of some chemical processes as a function of temperature:

$$K_1 = A \cdot B^{t1} \qquad \text{(IX)}$$

where K_1 is the reaction velocity at the temperature t_1, A and B constants of the equation. The relation between temperature

nd velocity thus appears as exponential. The same formula ıay also be written:

$$log\ K_1 = a + bt_1, \qquad (X)$$

hen we put $log\ A = a$ and $log\ B = b$. From this modification it evident that the velocity should increase in geometrical proression when temperature rises arithmetically. In graphical epresentation the logarithm of velocity plotted against temerature should yield a straight line.

As BERTHELOT's formula holds good only for narrow temperaıre intervals, VAN'T HOFF (1884) has proposed a new equation, ow generally called the VAN'T HOFF-ARRHENIUS law or ARRHENIUS w, which will be discussed in II. 7. By a simplification, permisble for temperatures not too far apart, this law takes the form BERTHELOT's equation (IX, X). Thus simplified the VAN'T OFF's law may be expressed as follows: When the temperature ses 10^0, the velocity of most chemical processes increases two three times. Hence the commonly known relation:

$$\frac{K_{t+10}}{K_t} = Q_{10} = 2 \text{ to } 3 \qquad (XI)$$

, in still another form which permits of calculating Q_{10} for any mperature difference:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{\frac{10}{t_1 - t_2}} \qquad (XII)$$

practice this equation is used in logarithmic transcription:

$$log\ Q_{10} = 10\ (log\ K_1 - log\ K_2)/t_1 - t_2 \qquad (XIII)$$

This method of mathematical treatment of the velocity of action in function with temperature was introduced into biology chemists, and the beginning of the biological problem of $_{10}$ actually may be found in chemical literature. In VAN'T OFF's lectures on physical chemistry (VAN'T HOFF and COHEN 96), CLAUSEN's results on plant respiration are analysed and $_{10}$ is stated to be equal to 2,5. Another mention of the Q_{10} biological processes appeared a few years later in COHEN's ok (1901), where among other data those of O. HERTWIG on e velocity of tadpole development are recalculated and values Q_{10} given. Also in the following years the biological question Q_{10} still remained in the hands of chemists. In 1903 ABERSON lculated the Q_{10} of the velocity of alcoholic fermentation and

the 1905 volume of the *Zeitschrift für Elektrochemie* contained several papers dealing with biological reactions in relation to temperature (Abegg, Cohen, Herzog). In all of them it was stated that the Q_{10} for biological processes lies between 2 and 3, as in the majority of chemical reactions.

The first biologist who used this method was Thunberg (1904) who found that Q_{10} of the respiration velocity of frog's muscle is about 2. But the chief advance was made by Kanitz, whose first paper on the subject (1905) appeared in connection with the above mentioned notes of certain chemists. From this moment onwards the number of biologists and of papers dealing with the question rapidly increased. Blackman (1905), Peter (1905), J. Loeb (1905), Snyder (1905) were among the protagonists of the new views.

It is noteworthy that some investigators, biologists and chemists, emphasized from the beginning the complex character of biological reactions and of living systems as compared with the relative simplicity of the inorganic systems (O. Hertwig 1898, E. Cohen 1901), but biologists have readily neglected this important feature of protoplasm and of protoplasmic activity.

Practically all the results which have been obtained in this field of investigation during the first quarter of this century have been summarised by Pütter (1914, 1927), Kanitz (1915, 1925), Matisse (1919) and Przibram (1923). In brief it may be said that the Q_{10} of the rate of biological processes lies in many cases between 2 and 3, in so far as temperatures not too close to nor too distant from the optimum are concerned. Also many biological reactions are known in which Q_{10} is much lower than 2. Generally at low temperatures the Q_{10} rapidly increases in value. This phenomenon of variation of Q_{10} value and its probable causes are discussed in the following paragraph.

5. Inconstancy of Q_{10} with temperature

It could not escape the attention of biologists at the beginning of this line of investigation that for one and the same process the value of Q_{10} varies with temperature (Snyder 1905). Originally these variations were attributed to imperfections of experimental technique. Therefore Robertson (1906), Snyder (1907), J. Loeb (1908), and others simply took the average of all values observed at different temperatures, and found that it lay between 2 and 3

et it soon became obvious that the inconstancy of Q_{10} with mperature was not accidental but was systematic, and that $_{10}$ generally increases in value in a progressive and regular way the temperature decreases. In many cases the Q_{10} became ren greater than 10 at low temperatures, when the same process ve $Q_{10} = 2$ or even less at the higher ones. It is now generally cognised that systematic variations of Q_{10} with temperature e a constant and general feature of biological temperature tion. FAURÉ-FREMIET (1925) gives a graph demonstrating e regularity with which Q_{10} varies with temperature. It is ly in a few experiments which PÜTTER (1914) called „Parade-rsuche" that the Q_{10} of a biological process remains unchanged er a wide range of temperature (see also NICLOUX 1931 *b*, *c*). Such nstancy is often, but not always, met with in those phenomena hose temperature coefficient is relatively low (many cases of xygen consumption), or in cells rich in water (FAURÉ-FREMIET 925). In a few other cases Q_{10} was found on the contrary to decrease value when temperature was lowered (see EPHRUSSI 1929).

The inconstancy of Q_{10} with temperature is usually explained five different ways:

(*a*) Q_{10} varies with temperature even in inorganic reactions, was already known to VAN'T HOFF, who explained the phenomenon by the variations in viscosity of the solvent with temperature. Many biologists think that the variations of Q_{10} in ology have the same cause and that Q_{10} *must* vary with temperature in both living and nonliving systems (KANITZ 1907, NYDER 1909, 1911*a*, 1931, ROGERS 1911, COHEN STUART 1911, UTGERS 1911). With regard to the claims which are still maintained, namely that Q_{10} *must* behave in this manner, it is advisable to compare the degree of discrepancy in living and nonliving systems (Table IV, p. 14).

It is evident that these variations are of less than 10 % in the se of inorganic reaction, but that they are greater than 100 % even 200 % in enzymatic reactions and in biological processes.

Some authors supposed that the sinking of Q_{10} with increasing temperature is due to the interference with reaction equilibria KANITZ 1907*a*, DEMOLL and STROHL 1909), or to the solubility liberation of molecules (KANITZ 1907*a*, LOEB and WASTENEYS 911, PRZIBRAM 1923), or to secondary factors in general NICLOUX 1931*c*).

Table IV

Interval	Q_{10}					
	Reaction between H_2O_2 + 2 HJ[1])	Activity of liver lipase[2])	Activity of a malt amylase[3])	CO_2 production in seedlings of pea[4])	Frequency of cat's heart[5])	Protoplasmic streaming in Vallisneria[6])
0—10°	2.080	1.50	3.2	3.0	—	> 4.0
10—20°	2.078	1.36	2.2	2.4	5.40	2.3
20—30°	1.940	1.26	2.0	1.8	2.15	1.6
30—40°	1.932	—	1.5	1.4	1.84	1.3
40—45°	1.920	—	—	—	1.35	—

(*b*) TAMMANN (1895) and DUCLAUX (1898—99) showed that the rate of enzymatic reactions increases on heating up to a certain optimum and then decreases, the enzyme being destroyed at high temperatures. BLACKMAN (1905) applied this principle to living matter and explained the inconstancy of Q_{10} with temperature on the assumption that the activity of living substance is slowly destroyed even at temperatures approaching the optimum. In reality the experiments of MATTHAEI (1905) demonstrated that the velocity of plant assimilation steadily decreases with time at temperatures lying close to the optimum. BLACKMAN's view, shared chiefly by PÜTTER (1914, 1927), however, was criticized by KANITZ (1915). The chief objection made by this author is that the decrease of activity with time could only be experimentally demonstrated at 25° and higher, whereas Q_{10} decreases in value from the lowest temperatures (—5°) onwards.

(*c*) KANITZ (1915) suggested that Q_{10} must necessarily deviate at and near temperatures which represent the limits of life and which thus act differently from temperatures which are "normal". According to KANITZ the beginning of enzymatic activity at low temperatures is connected with a high Q_{10}, as is

[1]) Calculated from HARCOURT and ESSON (1867).
[2]) „ „ KASTLE and LOEWENHART (1900).
[3]) „ „ ERNSTRÖM (1922).
[4]) „ „ KUIJPER (1910).
[5]) „ „ LANGENDORFF (1897).
[6]) „ „ VELTEN (1876).

so its destruction by high temperatures. There are however wo questionable points in this explanation, *i. e.* that the two xtreme temperatures arrest the enzyme action by two quite ifferent mechanisms, the arrest being reversible in the one case nd irreversible in the other, and secondly the variations of Q_{10} ith temperature are evident not only at or near the temperature mits, but at all biokinetic temperatures as well.

(*d*) The varying value of Q_{10} at different temperatures has d some biologists to suppose that every biological process is ased upon a "catenary series" of chemical and physical processes, ach of which has its own temperature coefficient and becomes ne "limiting factor" (Blackman 1905) or "master process" of ne whole at a definite temperature. This explanation, known as ne Blackman-Pütter principle, has not been demonstrated in ct. On the contrary, the progressive and regular decrease of Q_{10} with increasing temperature makes such an explanation oubtful. — There will be more occasion to discuss this view in hapter IV (2, 4).

(*e*) The most generally accepted view to-day as to the in-onstancy of Q_{10} with temperature is that the rule of van't Hoff oes not apply to the majority of biological reactions. W. M. Bay-iss (1918) remarks that a temperature constant, which varies ith one of the variables, is no true constant, and this applies the Q_{10} in the highest possible degree. The number of investi-ators who arrived at this conclusion is so great that only some f them may be mentioned: Filon (1911), Krogh (1914*a*, *b*), Varburg (1919), Fauré-Fremiet (1924, 1925), Pantin (1924), ucciante (1927*a*), Shelford (1927, 1929). Some other names nd other objections to the general use of Q_{10} in biology may e found in the papers of Bělehrádek (1926*d*, 1928, 1930*a*).

6. Corrected exponential formulae

In addition to the corrected exponential formula, which as applied by Stephens and Barrow (see Krafka 1921), in ne special case of developmental velocity in *Drosophila*, Janisch 925, 1928) has proposed a more generalized modification of the an't Hoff's rule which would also embrace temperatures eyond the optimum:

$$y = \frac{m}{2}(a^x + a^{-x}) \qquad \text{(XIV)}$$

y being time, m time at the optimum, x temperature in °C and a a constant.

But it is doubtful whether there is any advantage in combining two different processes, *i. e.* temperature action on the velocity and on the destruction of protoplasm by heat, in a unique quantitative law. As pointed out by Bodenheimer (1926), Martini (1928), Bělehrádek (1929, 1930), Andersen (1929*b*, 1930) and Maas (1933), the formula does not correspond to reality with sufficient accuracy and has been tested in too small a number of instances to allow of generalization. Besides, Janisch seems to neglect the fact that the velocity above optimum is not constant but varies in time. The constants of his formula therefore would necessarily vary with time of exposure. He further supposes that protoplasmic destruction takes place even at low temperatures, which has not been demonstrated (II. 5, *b* — see further in II. 8).

7. Arrhenius law

We have seen (II. 4) that the Q_{10} rule is a simplification, allowed only for small temperature intervals, of a more complex and more accurate law, first proposed by van't Hoff (1884), theoretically elaborated by Arrhenius (see Kanitz 1915) and now generally used in chemistry (for its history, see especially Glaser 1924). This law is expressed by the following equation:

$$K_2 = K_1 \cdot e^{2\left(\frac{1}{T_1} - \frac{1}{T_2}\right)} \qquad \text{(XV)}$$

in which K_1 and K_2 are two velocities at two temperatures T_1 and T_2 respectively, the temperature being taken in degrees of the absolute scale, e is the base of natural logarithms and μ a constant, called "thermal increment" or "temperature characteristic", which has the meaning of a temperature coefficient. The simplest way of calculating μ is:

$$\mu = 4{,}6\ (\log K_2 - \log K_1) \,/\, \frac{1}{T_1} - \frac{1}{T_2} \qquad \text{(XVI)}$$

This formula seems to hold good in chemical processes with more accuracy than the Q_{10} rule (Matisse 1919), though there are some instances in which μ is not quite constant.

The value of μ is supposed to denote the "heat of activat-n" of the process, *i. e.* the number of calories which are necessary transform one gram equivalent of inactive molecules of the acting substance into active ones. A more detailed discussion the meaning of this formula may be found in RICE 924).

This law was introduced into biology with the view to obtain ore comparable results than those yielded by the Q_{10} rule. s first application seems to have been made by ABERSON (1903) alcoholic fermentation, by SNYDER (1905) to the heart beat the terrapin, by MADSEN and NYMAN (1906) to the killing of acterial spores by $HgCl_2$, by SNYDER (1907*a*) to the rate of nduction in nerves, by ROBERTSON (1908) to the respiratory ovements in frog, by ARRHENIUS (1908, 1915) to certain sero-gical reactions and some other biological phenomena. Of late e formula has been applied anew in the work of MATISSE (1919, 921), but chiefly in that of CROZIER and of his collaborators CROZIER 1924—1926; CROZIER and collaborators 1924—1931; e further BLISS 1926; L. A. BROWN 1926, 1928, 1929*b*; CASTLE 928; COLE 1925, 1926, 1929; COLE and ALLISON 1929; FEDE-GHI 1929, 1930; FRIES 1926; FULTON 1926; GLASER 1925; RAY 1928*b*; HECHT 1926, 1928; HELLER 1930; M. IRWIN 1925; cCUTCHEON and LUCKÉ 1926—1927; MILLER 1929; MITCHELL 929; MURRAY 1926; NAVEZ 1928—1931; ORR 1925; PINCUS 1931; ICHARDS 1926—1928; SHAPLEY 1924; SNYDER 1931; STEINER 932; STIER 1933; STIER and PINCUS 1928; STIER and WOLF 1932; ANG 1931*a*, *b*; WELSH 1932; WICKWIRE, SEAGER and BURGE 929; WORLEY 1933; YAMAMOTO 1931; etc.

In this chapter the theoretical implications of the ARRHE-US law will be put aside, a discussion in full being reserved r Chapter IV (4). Yet it is necessary to point out one or two teworthy features of the formula.

First it is useful to keep in mind that there is a close re-tionship between μ and Q_{10}, as the latter may be derived from e former by simplification for moderate intervals of temperature. his relationship was clear to former investigators (ROBERT-N 1908, 1920; COHEN STUART 1911), but is neglected by ROZIER.

ROBERTSON (1920) gives the following table of Q_{10} and rresponding values of μ:

Table V

Q_{10}	μ
2	13,200
10	44,000
100	88,000
1000	132,000
10,000	176,000

Following the Q_{10} rule, the logarithm of velocity gives a straight line when plotted against the ordinary temperature (^{0} C), while following the law of ARRHENIUS the logarithm of velocity gives a straight line when plotted against reciprocal value of the absolute temperature. But BĚLEHRÁDEK (1928) has pointed out that the reciprocal of absolute temperature is, as far as the biokinetic zone is concerned, practically a linear function of the ordinary temperature, which means that there is no practical difference between μ and Q_{10} in biology (fig. 6). Also PÜTTER (1927) has opposed the general use of the law of ARRHENIUS in biology for similar reasons. There are furthermore some objections of biological character to be made to the use of μ (BĚLEHRÁDEK 1928), the chief of which is that vital action is arrested at or in the

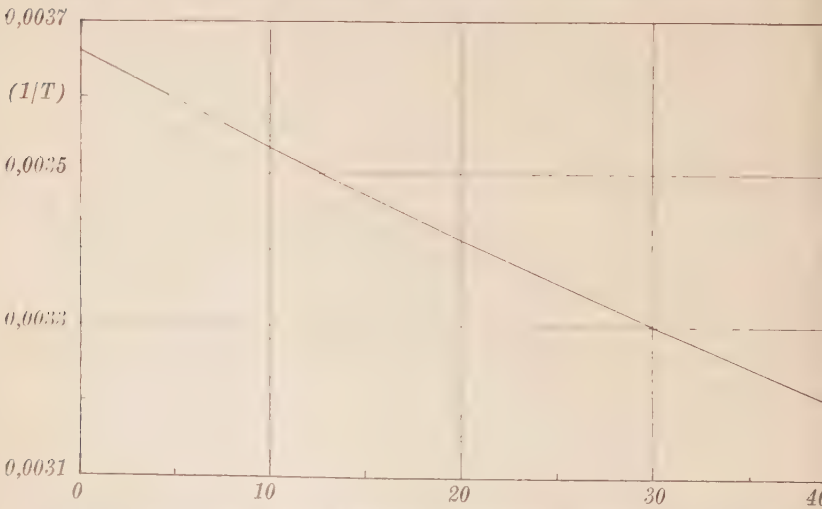


Fig. 6. Reciprocal of the absolute temperature (ordinate) is practically linear function of the ordinary temperature (abscissa), between 0^0 and 40^0. (BĚLEHRÁDEK 1928*b*).

cinity of 0°C, and not at —273°, so that the use of the absolute mperature scale is a superfluous complication.

As a matter of fact it was known even to those who were st to test the ARRHENIUS formula in biology, that the constant varies with temperature (ROGERS 1911, COHEN STUART 1911). rom what has just been said it is clear that it must vary with mperature in every case where Q_{10} does. Yet CROZIER and his -workers (1924—1931) attempted to give to these variations theoretical meaning in saying that different values of μ found different temperatures, belong to various individual biochemical catalytic processes upon which the complex "biological pro-ss" is based. This point of view is evidently incorrect, as monstrated by HEILBRUNN (1925), BĚLEHRÁDEK (1928*b*, 1929, 30), D. LUDWIG (1928*b*), FULMER and BUCHANAN (1929). On e contrary NAVEZ (1931) has defended CROZIER's view. The tails of this discussion must be read in original papers (see also ĚLEHRÁDEK 1932*b*). Besides that, the following authors find at the variations of μ with temperature are systematic and not dden: BĚLEHRÁDEK (1926*a*), FRIEND (1927), EMERSON (1929), URBATOV and LEONOV (1930), WALTON (1930), D. E. S. BROWN 930*b*), HAINES (1931), etc.

The constant μ decreases with increasing temperature also simple enzymatic processes studied *in vitro* (EULER and LAURIN 20, ERNSTRÖM 1922; numerous examples are found in KUHN 24, p. 152; see also HALDANE 1930, p. 65, and BĚLEHRÁDEK 30*a*, p. 38–39). EULER and LAURIN even give an equation from hich it is possible to calculate μ for various temperatures. It ould therefore be surprising that μ should be more constant *in vivo*.

In a few instances where a unique μ is found for all tem-ratures, Q_{10} also is constant in value, and if there are any fferences, they do not exceed the usual limits of unavoidable ror in biology. For the great majority of biological processes, wever, there are many reasons leading one to expect that μ ll not be constant with temperature.

8. Temperature coefficient *b*

In the lack of any rational temperature formula in biology, ĚLEHRÁDEK (1926*a*–*d*, 1927, 1928*a*, 1929, 1930*a*, 1931*a*–*c*) has oposed an empirical equation, which is:

$$y = \frac{a}{t^b} \qquad \text{(XVII)}$$

y being time, t temperature, a and b constants, the latter of which has the meaning of a temperature coefficient. When expressed in logarithms, the formula becomes:

$$log\ y = log\ a - b \cdot log\ t \qquad \text{(XVIII)}$$

from which it is clear that the logarithm of time (or of velocity), plotted against the logarithm of temperature, should give a straight line. Figures 7 and 8 are graphical representations of this relation. (Compare also figs. 11—13, 16, 17, 23, 26, 27).

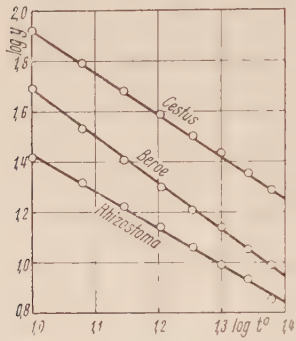


Fig. 7. Temperature action on the rate of O_2-consumption in certain marine animals (Vernon 1895 *a*). Abscissa: *log* of temperature. Ordinate: *log* of time required to consume a definite volume of O_2.

The exponent b is calculated as follows:

$$b = \frac{log\ y_1 - log\ y_2}{log\ t_2 - log\ t_1} \qquad \text{(XIX)}$$

or, because velocity (v) is the inverse of time (y):

$$b = \frac{log\ v_2 - log\ v_1}{log\ t_2 - log\ t_1} \qquad \text{(XX)}$$

According to the author, the formula holds good in many cases, yielding a unique b for all temperatures examined. Yet it cannot be said that it would hold good in every case. Crozier

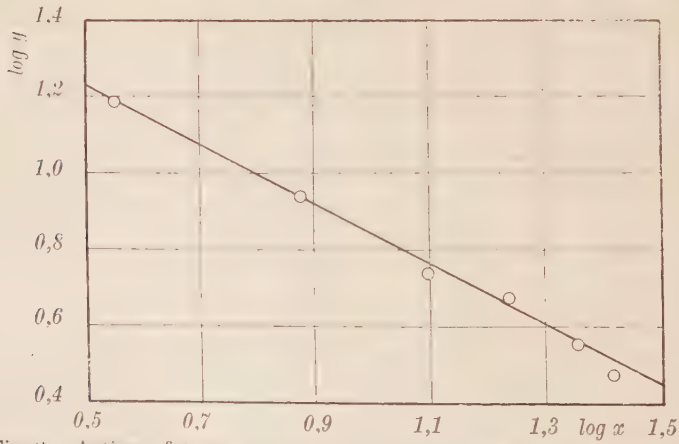


Fig. 8. Action of temperature on the rate of nervous conduction in the frog sciatic nerve (Rosenberg and Sugimoto).

d STIER (1927*a*) have shown that it does not agree with the perimental results of FRIES on the heart beat of the cockroach, here a unique μ for all temperatures could be found. Though ĚLEHRÁDEK (1929) showed that in this special case no definite nclusions can be drawn owing to the imperfections of experimental technique, there is no reason to expect the formula hold good in every instance.

From the equation (XVII, VIII) it may be seen that at C the reaction should be stopd. Yet this is not always the se. BĚLEHRÁDEK therefore inoduced into his formula a new nstant (α) which denotes the mperature threshold, from which e temperature is reckoned and ich coincides with the "biogical zero" for the process ncerned. Thus modified, the mula may be written:

$$y = \frac{a}{(t-\alpha)^b} \qquad \text{(XXI)}$$

Figures 9 and 10 are exples of this modified relation.

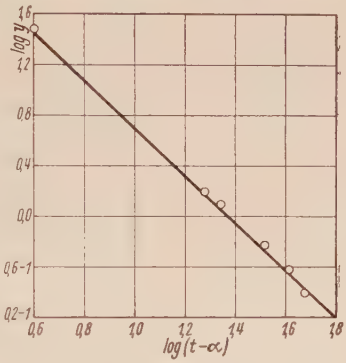
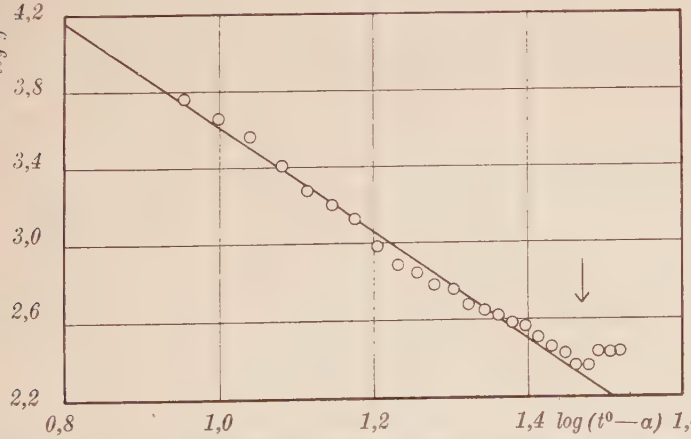


Fig. 9. Action of temperature on the rate of photosynthesis in the leaves of *Prunus laurocerasus* (MATTHAEI). Abscissa: *log* of time required to produce 1 mg of oxygen. Ordinate: temperature reckoned from -10^0 ($\alpha = -10^0$), in logarithmic values.



. 10. Action of temperature, reckoned from $+12^0$ ($= \alpha$), upon the ation of the prophase in chick myoblasts (BUCCIANTE 1927). Temature (abscissa) and time (ordinate) are represented logarithmically.

For many organisms, whose reactions are arrested in the vicinity of 0° C, it is obviously unnecessary to introduce α into the computation.

NAVEZ (1929) thinks that the equation fits with some exactness only such series of observations, where no abrupt change in μ occurs. It is thus important to show that a single value for b may be found in many cases in which Q_{10} and μ show a decrease with rising temperature. This fact, which strongly supports sceptical views as to the idea of CROZIER (see II. 7 and IV. 4) is illustrated by figs. 11 and 12 (compare also ROOT 1932). Also

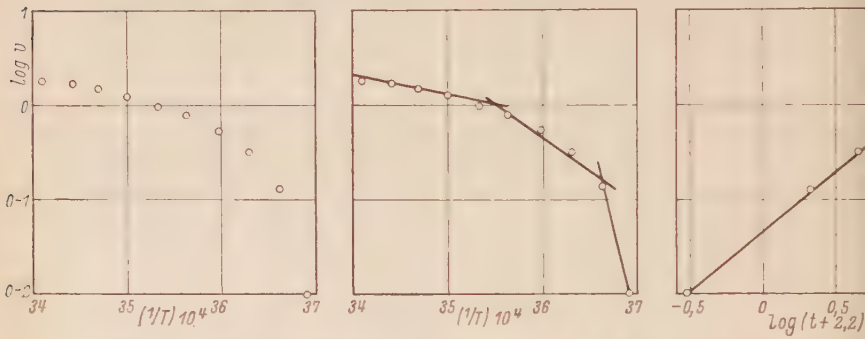


Fig. 11. Velocity of ameboid movement as a function of temperature (PANTIN). In the left: *log* of velocity, plotted against the inverse of absolute temperature, gives a regular curve. — In the middle: the same series of points as in the left, three straight lines being drawn so as to fit best the observed data, on the assumption that the law of ARRHENIUS holds good in this case and that the movement is based upon three distinct "master processes". — In the right: *log* of velocity plotted against *log* of temperature, the latter being reckoned from —2,2°. One single line suffices to represent the relation.

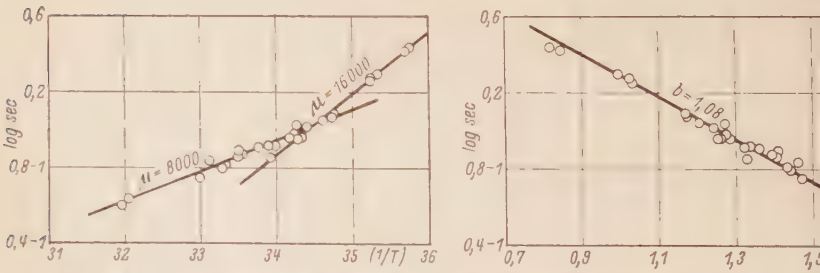


Fig. 12. Velocity of locomotion in *Paramaecium* as influenced by temperature (GLASER 1924). Left side: in the representation required by the formula of ARRHENIUS. Right side: as required by the formula of BĚLEHRÁDEK.

ases in which JANISCH (1925) applied his corrected exponential equation (see II. 6), are represented with sufficient accuracy by he formula, as is evident from the fig. 13.

The exponent b generally has a value between 0.6 and 4.0. In most cases it is equal to 1.0—3.0. When it equals 1, the formula becomes:

$$y = \frac{a}{t} \qquad \text{(XXII)}$$

or:

$$y \cdot t = a \qquad \text{(XXIII)}$$

which is a formula identical with that of thermal summation and of the linear relation between temperature and velocity

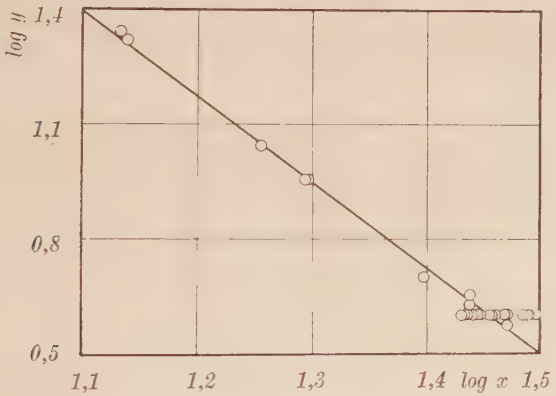


Fig. 13. Duration of the embryonic development of a moth, *Ephestia kühniella*, in function with temperature (JANISCH 1925). Both axes are logarithmic.

III, VIII). The rule of thermal sums and linear relation are thus only special cases of a more general relation between the temperature and the rate of biological processes (BĚLEHRÁDEK 1929, 1930*a*).

L. HILL and EIDENOW (1924) found that the rate of killing of infusoria by ultraviolet rays depends on temperature thus:

$$t \cdot \sqrt{T} = \text{Const.}, \qquad \text{(XXIV)}$$

t being temperature in degrees Centigrade, and T time of exposure. The same equation may also be written as follows, if one puts $T = y$ and $(\text{Const.})^2 = a$:

$$t^2 \cdot y = a \qquad \text{(XXV)}$$

or:

$$y = \frac{a}{t^2} \qquad \text{(XXVI)}$$

This equation again is only a special case of the formula discussed here, b being equal to 2.

The formula is identical with that suggested by Porodko (1926*b*) for the rate of killing of organisms by heat (see X. 5, *c*). According to Rönne (1930) this type of equation seems to hold good also for many other biological phenomena outside the temperature action.

9. Other temperature formulae

Veley and Waller (1910) applied Esson's formula, originally proposed for certain chemical reactions, to the rate of action of certain drugs on muscle at different temperatures. The formula is:

$$\frac{K_2}{K_1} = \left(\frac{T_2}{T_1}\right)^m \qquad \text{(XXVII)}$$

where K_1 and K_2 are two velocities at temperatures T_1 and T_2, the absolute temperature scale being used, and m is a constant. The practical value of this formula has not yet been demonstrated in many cases in biology, though Dhar (1920) recommended its more general use. When the absolute temperature is replaced by the ordinary one, the formula is changed into the one discussed in the preceding paragraph.

A. G. Mayer (1914) found that in some cases of nervous conduction in the medusa the rate is proportional to the square of the temperature reckoned from the optimum downwards. Neither was this relation sufficiently demonstrated in biology.

Formulae describing the effect of high temperature will be dealt with in Chapter X.

CHAPTER III

VARIATION OF TEMPERATURE COEFFICIENTS WITH EXTERNAL AND INTERNAL FACTORS

1. General remarks

Before attempting to compare temperature coefficients it s useful to know whether they are constant or not when the conditions of the reaction are varied.

It is known that in inorganic reactions the temperature coefficients vary with the nature of the solvent, as first demonstrated by v. HALBAN (1909) in dissociation of triethyl-sulfinbromide:

Table VI

Solvent	μ	Q_{10}
Amylalcohol	16.700	4.14
Propylalcohol	16.970	4.24
Benzylalcohol	17.140	4.30

Other similar examples are found in SKRABAL (1916). One f the most interesting is that the presence of some electrolytes nodifies the Q_{10} of certain saponifications. Most of the variations uoted by SKRABAL are not very important, but it may also appen that a process, which normally ocurrs with a positive emperature coefficient, may show a negative one under the ction of external conditions (see also RICE and UREY 1924).

More important variations are however known to occur enzyme action. Concentration in substratum affects the value Q_{10} in some enzymes (KUHN 1924). LUIPPOLD (1929) has emonstrated that pH variations greatly affect the value of Q_{10} an amylase (fig. 14). Similar effects are known also from the ork of NELSON and BLOOMFIELD (1924) on saccharase. ERNSTRÖM

(1922) found analogous variations of μ in amylase in the presence of NaCl. It may be that differences in Q_{10} and in μ reported by various authors in one and the same enzyme are based on such effects (see HALDANE 1930).

In the process of diffusion the temperature coefficient varies with the nature of the diffusing substance and with the rapidity of the diffusion itself, as stated by KOHLRAUSCH (1908) and ÖHOLM (quoted from H. S. TAYLOR 1931). The more rapid the diffusion of a given substance, the lower is the temperature coefficient (see also IV. 9).

Fig. 14. Temperature coefficient (Q_{20}) of the activity of an amylase varies with the *pH* of the medium (constructed from data of LUIPPOLD 1929).

There are therefore many reasons to suppose that temperature coefficients of biological processes will similarly be affected by variations in the external conditions as well as by those of the internal state of living systems themselves. It will be seen later on (IV. 9) that an attempt may be made to understand many of these variations from one single standpoint.

2. Effect of varying water content

It is known that in some organisms the water content varies with the degree of relative humidity in the air. Especially in arthropods it is easy thus to modify the water content of their bodies to a large extent. As far as data are available on the rate of insect development under varied temperature and humidity, temperature coefficients at various water content of the body may be calculated. BĚLEHRÁDEK (1926*c*, 1930*a*, 1932*b*) gives such figures of which the most demonstrative are those computed from SHELFORD's (1927) data on the rate of development of the coddling moth (*Carpocapsa pomonella*) under varying conditions. From these figures (fig. 15) it is evident that both μ and Q_{10} vary systematically with the humidity, being high at low humidities, decreasing at higher ones and increasing again at the

highest relative humidities. — See further Bělehrádek and Mládek (1934) for O_2 consumption in larvae of insect.

From Andersen's data (1930) on *Sitona lineata*, it follows that the temperature coefficient of the rate of development rather decreases with decreasing humidity. Similar behavior of Q_{10} is shown in Titschack's figures on the velocity of development of the moth (*Tineola bisselliella*), yet the number of observations here is too small to allow of a conclusion. From Wardle's (1930) results on the development of the blow-fly (*Lucilia sericata*) no such variations in temperature coefficient are noticed.

Fig. 15. Temperature coefficient Q_{10} (empty circles) and μ as infleunced by atmospheric humidity, in the case of insect development (Shelford's data).

3. Effect of electrolytes

From E. G. Martin's results (1904) on the rhythm of isolated heart of terrapin at various temperatures and at different concentrations of KCl in the perfusion liquid, the following figures have been calculated (Bělehrádek 1932*a*):

Table VII

% KCl	*b*
0	0.84
0.003	3.12
0.08	2.80
0.11	4.70

Potassium chloride thus increases the temperature coefficient (compare also fig. 16) of the heart beat. Analogous variations in Q_{10} have been found by J. J. Bouckaert, J. P. Bouckaert and Noyons (1922) also in the frog's heart in relation to the Ca content of the perfusion liquid.

Sherwood and Fulmer (1926) and Fulmer and Buchanan (1929) reported that the value of μ in yeast growth depends on the concentration of NH_4Cl in the medium. Cerighelli (1926*a*, *b*) studied the effect of various $CaSO_4$ concentrations upon the

temperature coefficient of root growth in the pea with a similar result. From the observations of R. Meyer (1928) it may be concluded that $(NH_4)_2SO_4$ influences the value of Q_{10} in the growth of *Aspergillus niger*.

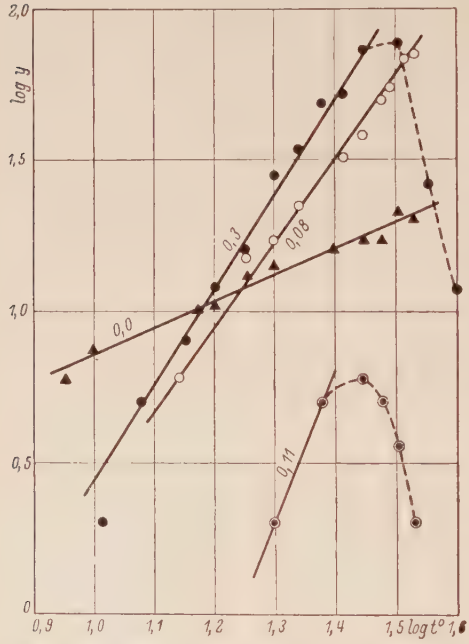


Fig. 16. Heart beat frequency (in logarithms, ordinate) as dependent on temperature (in logarithms, abscissa), according to data of E. G. Martin. Concentrations of KCl marked on the respective lines.

As to the modifications of temperature coefficients under the action of acids, alkalis and of varying pH, A. J. Clark's (1921) experiments on perfused frog heart seem to indicate that Q_{10} is higher on the alkaline side than at neutrality, yet no systematic studies have been undertaken to clear up this point. As far as may be judged from the scarce data of Friedheim and Runkhelman (1930), Q_{10} of glucose consumption in the explants of the chick heart decreases when pH of the medium is lowered. From the results of W. Brown (1922) on the temperature action in the rate of growth of *Penicillium glaucum* under varied CO_2 content the following figures are computed:

Table VIII

Atmosphere	Q_{5-15}
Normal air	4.25
Air + 10% CO_2	5.0
„ + 20% CO_2	6.0

It may thus be concluded that various electrolytes greatly modify the temperature coefficients of many biological processes.

4. Effect of narcotics

KOIKE (1911) determined the rate of propagation of the nervous impulse at different temperatures in normal and anaesthetised nerve, but his figures are too irregular to allow a conclusion. FREUND (1922), working under BABÁK's direction, could not detect any change in Q_{10} of the heart rhythm on addition of ethyl alcohol, but D. E. S. BROWN (1930) reports that alcohol lowers the value of μ in excised frog's heart. COLE (1925) thinks that chloretone does not modify the value of μ of the frequency of the pulsating vacuole in Paramaecium. But BĚLEHRÁDEK (1926*c*) has pointed out that the analogous results of KHAINSKY (1911), gained without narcosis, yield a much lower b than COLE's figures with chloretone.

The question thus remains open as to whether narcosis modifies temperature coefficients in biology, and in which direction.

5. Effect of other substances

It is known since SLATOR's (1908) investigations on the rate of CO_2 production in yeast that the temperature coefficient of the process varies with the chemical nature of the sugar used:

Table IX

Sugar	b of CO_2 production
Galactose	1.40
Dextrose, levulose . .	1.86
Saccharose, mannose .	
Maltose	2.52

These results are represented graphically in fig. 17. Similarly n the respiratory process of *Phycomyces*, the temperature coef-

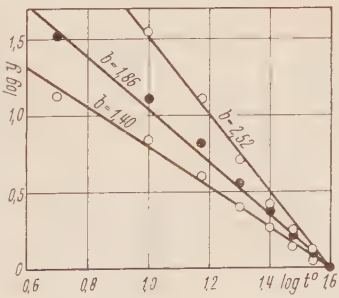


Fig. 17. SLATOR's (1908) figures on the rate of alcoholic fermentation as influenced by temperature, in bilogarithmic representation (abscissa = *log* of temperature, ordinate = *log* of time, time being taken as reciprocal of the velocity). Upper line: maltose. In the middle: levulose. Lower line: galactose. Respective temperature coefficients (b) marked on the graphs.

ficient (b) varies with the medium, as may be found from De Boer's (1927) figures:

Table X

Medium	b of O_2 consumption	b of the CO_2 production
Linseed . .	1.34	1.34
Bread . .	1.04	1.40

Besides the effects of electrolytes and of narcotics, which have just been discussed, some other substances seem to modify the values of the temperature coefficients of the heart rhythm, as is evident from Gellhorn's (1924, 1926) experiments with tyramine and adrenaline. Bělehrádek (1932*a*) has found that caffeine in small doses increases, in greater ones decreases b of the rhythm of isolated frog's heart:

Table XI

Per cent of caffeine	b of the rhythm
0	1.15 ± 0.09
0.133	1.26 ± 0.02
—	—
0	1.30 ± 0.04
0.015	1.57 ± 0.08
0.1	1.10 ± 0.07
—	—
0	1.25
0.03	1.35
0.06	1.39
0.09	1.21

According to Bělehrádek and Mirski (1932), small amounts of guanidine increase the b of the heart rhythm in the frog. Freund (1922) failed to see any such modifications under the action of cardiotropic alkaloids. Also Barcroft and Izquierdo (1931) have obtained negative results with atropine.

Strychnine greatly lowers the temperature coefficient of the duration of muscular action currents (Lorenz 1926). Ramaglia (1929) reports that Q_{10} of oxygen consumption in the toad is

lowered by injection of guanidine. Also ingestion of glucose seems to modify the μ of heart frequency in Limax maximus, as shown by CROZIER and STIER (1926*a*).

According to EMERSON (1929), different chlorophyll concentrations which he artificially varied in cells of *Chlorella* are not associated with differences in temperature action upon the rate of photosynthesis.

6. Effects of varied O_2 pressure

BABÁK and ROČEK (1909) found that Q_{10} of respiratory movements in the larvae of Libellulids is 1.8 in aerated water but that it increases to 2.2 under lack of oxygen. Also PÜTTER (1914) showed that the Q_{10} of oxygen consumption in the frog and in the leech varies with the partial pressure of O_2 (see also CROZIER and STIER, 1926*a*). In experiments of BABÁK and KŮHNOVÁ (1909) no significant differences in the Q_{10} of respiratory rhythm in urodeles could be detected when the O_2 pressure was varied. From the more recent investigations of MACK (1930) on CO_2 production in wheat seedlings the effect of varied O_2 pressure upon the value of the temperature coefficient may be computed as follows:

Table XII

Per cent of O_2	b
0.6	1.40
3.1—50.0	1.65
90—98.3	1.78

7. Effect of concentration of the reactants

In enzyme action and in the diffusing process the temperature coefficients depend on the concentration of the reacting or diffusing substances respectively (III. 1). Besides the effect of different O_2 pressure, which has been analysed in the preceding paragraph (III. 6), some isolated records may be found in biological literature regarding other substances as well.

CROZIER (1922) examined the rate of penetration of HCl into living cells of the nudibranch *Chromodoris zebra* and

found that it depended on temperature and on concentration as follows (see also fig. 18):

Table XIII

Concentration of HCl	Q_{10} (above 18°) of the rate of penetration
0.0063 n.	1,19
0.01	1,68
0.10	1,81
0.33	1,89

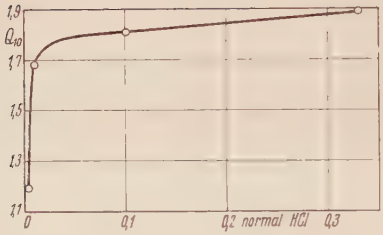


Fig. 18. Q_{10} of the rate of penetration of HCl into certain animal cells increases with the concentration of acid (Crozier 1922).

The rate of accumulation of brilliant cresyl blue in *Nitella* shows the following relation to concentration and temperature (M. Irwin 1925):

Table XIV

Concentration outside	Q_{10}	μ
0.00001 mol.	5.9	30.900
0.00002 „	4.4	26.000
0.00004 „	4.5	26.200
0.00008 „	4.7	—

From the results of Mac Arthur and Baillie (1929*b*) it appears that the temperature coefficient of survival of *Daphnia* in a medium containing KCN decreases with increasing concentration (see the table XVI on p. 33).

In spite of the above described effects of the concentration upon the magnitude of temperature coefficients, no definite rule can be laid down as to the trend of such changes.

8. Effect of light

From a certain theoretical point of view, which will be discussed further on (IV. 9, *f*) it is important to know if the temperature coefficients of biological processes are varied by absorbed radiation. In spite of the scarcity of available information, it seems that this is so at least in some cases.

Vallot's (1921) results on the rate of reduction of methylene blue by animal tissues show that the temperature coefficient is higher in darkness than in light (fig. 19):

Table XV

	b
In obscurity. . .	3.08
In sun	2.24

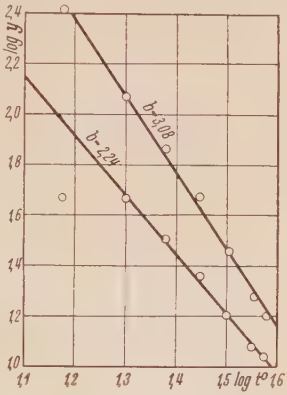


Fig. 19. Action of temperature (in logarithmic values, = abscissa) upon the rate (expressed in logarithms of times, = ordinate) of the reduction of methylene blue by pulped mammalian tissue. Upper line: in dark. Lower line: in solar light. (Constructed form data of Vallot.)

Crozier and Stier (1926*b*) suggest that abnormally low values of μ for speed of movement of *Oscillaria* might be explained by the action of light, and also Navez (1928) admits this. Hinrichs (1930) observed that the Q_{10} of the heart rhythm in *Fundulus* decreased slightly on irradiation by ultraviolet rays.

From data obtained by Hoagland, Hibbard and Davis (1926) on the rate of accumulation of bromine in the cell-sap of *Nitella* under varied temperature and illumination, the following values of Q_{10} are calculated:

Table XVI

No. of experiment	Q_{10} in darkness	Q_{10} in light
1	1.53	2.95
2	1.74	2.86
3	1.45	2.50
—	—	—
Average	1.57	2.77

From the results of Magistrits and Schäfer (1930) on the velocity of ecdysis of phosphatids and of sugar in plants it appears that temperature coefficients decrease on illumination, especially on application of ultraviolet light.

In photochemical reactions the value of temperature coefficients often varies with the wave length and also with the intensity of radiation. This special point is to be analysed in more detail in another section (IV. 5).

9. Mechanical effects

In transmission of action currents in the muscle of the frog, the temperature coefficient seems to vary with the elastic tension of the tissue (Hieronymus 1913).

10. Seasonal variations

in temperature coefficients have been analysed by Crozier and Stier (1925*b*) in molluscs and by Barcroft and Izquierdo (1931) in the heart of the frog. They may be conceived, following Bělehrádek (1931*a*), as being associated with thermal adjustment (see III. 15).

11. Age differences

Bialaszewicz (1908) seems to have been the first to point out that Q_{10} of developmental rate increases with the stage attained. In 1912 Cesana found that the Q_{10} of the heart beat frequency in the chick embryo increases with age. Murray (1926) obtained with the same object data which clearly show a regular variation of μ with the age of the embryo. While the values of μ for the ventricular beat increase with age, those for the auricular beat vary in the opposite direction (fig. 20). Similarly more recent analogous investigations made by Cohn (1928) show a regular decrease of temperature coefficient with the age.

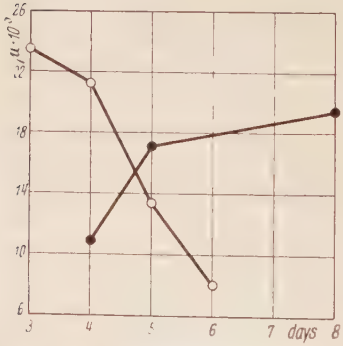


Fig. 20. Variation of the temperature characteristic μ of the heart beat frequency in chick embryo with age (= abscissa). Empty circles: auricle. Black circles: ventricle. (Data of Murray 1926.)

As to the heart beat of other species, an increase of temperature coefficient with age was demon-

strated by LAURENS (1914) on larvae of *Amblystoma*, by ANDERSEN (1929*a*, *b*) on the lizard and on larvae of the trout, by CROZIER and HUBBS (1924) on *Leucichthys artedi* (a fish). All these examples are analysed in more detail in a paper by BĚLEHRÁDEK (1930*a*). CROZIER and STIER (1927*b*) have also found different μ values in embryonic and adult heart of *Limulus*, and GLASER (1929) has confirmed such differences in the heart of *Fundulus*.

As regards the velocity of development, BĚLEHRÁDEK (1926*a*—*c*, 1928*b*, 1930*a*) has reviewed the earlier and more recent bibliography from this standpoint and could show that different stages of development often are associated with different temperature coefficients, and that these differences are systematic in function with age. According to a quotation from LUDWIG (1928*b*), it was noticed as early as 1910 by SANDERSON that μ of insect development varies with the age. There is now strong evidence to support such an idea, furnished by LOEB and NORTHROP (1917), BONNIER (1926), FRIEND (1927), LUDWIG (1928*b*), MELVIN (1928), DE BOISSEZON (1930) and by other entomologists. But by far the most demonstrative are the results of BLUNCK (1924) on the development of *Dytiscus semisulcatus*, as recalculated by BĚLEHRÁDEK (1926*c*, 1927, 1929, 1930*a*). Fig. 21 shows the relation between the velocity of development and temperature in the bi-logarithmic system (as required by formula XVII discussed in II. 8), whereas fig. 22 demonstrates that the variations of b with age describe an S-shaped curve. Likewise CROZIER (1926*a*) has analysed BLUNCK's data by means of the ARRHENIUS formula, but he was unable to detect any variation of μ with age. This supports the view that the formula does not yield good results in biology (see II. 7). — In 1926 (*c*) BĚLEHRÁDEK suggested that b often suddenly drops during the metamorphosis in insects, yet more recently he has changed his view (1930*a*) and thinks that this cannot yet be sufficiently demonstrated.

Variations of temperature coefficients with age in the development of Amphibians are not evident from the often quoted experiments of O. HERTWIG (1898) because of their irregularity. Yet from analogous results of KROGH (1914*a*) it may be concluded that the temperature coefficient tends to increase with age (see BĚLEHRÁDEK 1926*c*). — Also FAURÉ-FREMIET (1925) claims that

the Q_{10} values of developmental processes in general are unlike for different stages (see also PETER 1905).

Similar age differences seem to exist in temperature coefficients of the rate of metabolism. BODINE (1929) has observed that as the egg of certain Orthopters develops, the response of its metabolism to temperature becomes progressively greater.

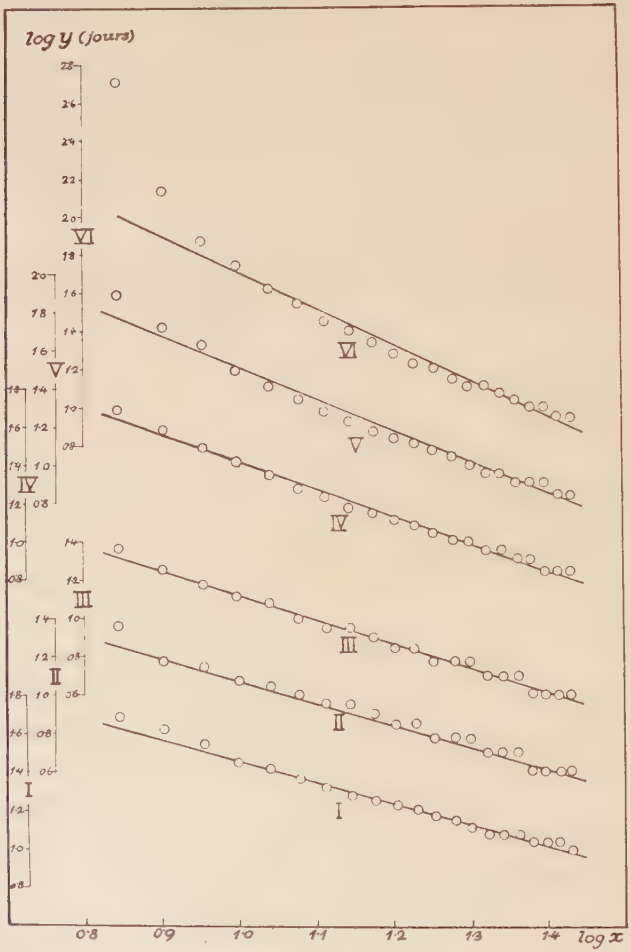


Fig. 21. Duration (*log* of the number of days, = ordinate) of the successive developmental stages of *Dytiscus*, in function of temperature (in logarithms, = abscissa). Increasing slope of the lines indicates increasing temperature coefficient. (From BĚLEHRÁDEK 1929, according to data of BLUNCK.)

Bělehrádek (1930*a*) has shown from the results of Battelli and Stern (1913*b*) that *b* of oxygen intake and of CO_2-production in different developmental stages of *Bombyx mori* are unlike, as

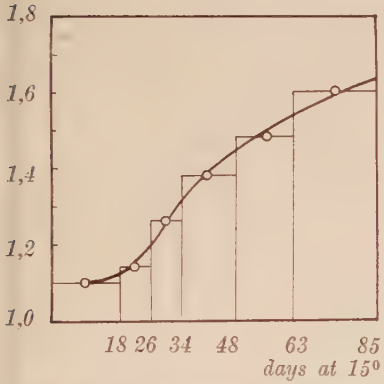


Fig. 22. Temperature coefficient *b* (= ordinate), computed from the fig. 21, increases regularly with the age attained (= abscissa). (Bělehrádek 1929.)

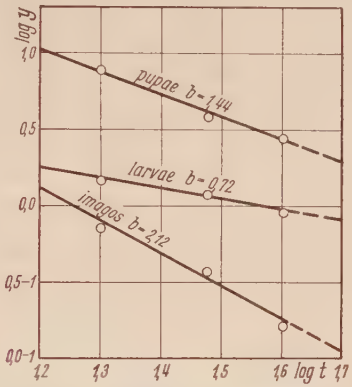


Fig. 23. Action of temperature (abscissa) on the rate of O_2-consumption in different developmental stages of the silkworm (*y* = reciprocal of the rate). (Battelli and Stern 1913*b*.)

is clear from fig. 23. Also from Bodenheimer's (1930) figures on the respiratory metabolism in the desert locust (*Schistocerca gregaria*) it appears that *b* varies as a function of age:

Table XVII

Stage	*b* of respiration
Embryonic	0.89
First larval instar . . .	1.38
Third larval instar . . .	1.52
Fifth larval instar . . .	2.40
Young imago	1.90—2.02
Old imago	2.20

Relatively little is known about similar variations in plants. Yet Huber and Nipkow (1923) have observed that the rate of excystation in *Ceratium hirundinella* depends on the age of cysts and on temperature, and from their figures it is evident that the

Q_{10} varies systematically with age (fig. 24). Bělehrádek and Bělehrádková (1929) have shown that the rate of oxygen intake in a fern, *Scolopendrium scolopendrium*, gives a *b* varying with the age of the leaves[1]).

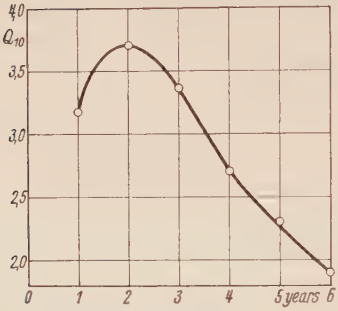


Fig. 24. Q_{10} of the rate of excystation in *Ceratium* as influenced by the age of the cysts (abscissa). (Huber and Nipkow.)

As pointed out by Bělehrádek (1931*c*), no significant age differences in temperature coefficients are found in processes which themselves show a low temperature coefficient. Thus he was unable to detect any such difference in the oxygen consumption of embryos and young of *Carcinus maenas* and in that of young and adult aphids (*Macrosiphum pellargonii*). It seems also that the electric conductivity of young and adult tissues responds to temperature variations in the same way.

12. Sexual differences

Nobody as yet has attempted to examine systematically whether the temperature coefficients of biological activities show sexual differences. Yet such differences are apparent from the work of Bonnier (1926) on the development in *Drosophila*, while Bliss (1926) could not detect any in the duration of the prepupal period in the same insect. According to Alpatov and Pearl (1929) the duration of life in the female *Drosophila* is modified by temperature to a greater extent than in the male:

---

1) Variations of temperature coefficients with age in general have been criticized by Crozier and Stier (1927*a*) who state "the notion that the temperature coefficients of a given activity decrease or increase regularly with the age of the organism is simply untrue". It is difficult to conceive such a point of view in the presence of so many facts. Similar criticism has been offered also by Navez (1931) in connection with Crozier's objections. A detailed answer has been published by Bělehrádek (1929, 1932*b*).

Table XVIII

	Q_{10} (average)
Male	1.92 ± 0.04
Female . . .	2.31 ± 0.07

Experiments of MacArthur and Baillie (1929*a*, *b*) demonstrate sexual differences in temperature action upon the heart rhythm, the duration of life and the susceptibility to KCN in *Daphnia magna:*

Table XIX

	b in male	*b* in female[1])
Heart beat	1.26	1.10
Life duration	1.31	1.10
Killing in KCN, 0.001 m.	1.36	1.20
Killing in KCN, 0.002 m.	1.20	0.80

Stumper (1922) found in the locomotion of ants (*Messor barbatus*) a $Q_{10} = 1.78$ in the worker and 1.95 in the apterous female.

At least two of these instances, namely the experiments of Alpatov and Pearl and those of MacArthur and Baillie, lie beyond the limits of experimental error and they show definitely that the temperature coefficient of one and the same reaction may be unlike in the two sexes.

13. Regional differences in temperature coefficients of organ activities

Moorhouse (1913) reported that the percentage degree of acceleration on heating is higher in the beat of coronary and septal strips excised from a cat's heart than in the rest of the heart tissue. Murray (1926) ha sdemonstrated that μ of the heart beat frequency in the chick embryo is unlike for ventricular and auricular activity. His figures are reproduced above (p. 34, fig. 20).

1) The authors give values of Q_{10}, which, though inconstant with temperature, show analogous sexual differences. Yet it seems simpler to use *b* in this case, as its value does not change with temperature.

Filon (1911) found that temperature affects the excitability (chronaxie) of different muscles of the same species (green frog) to a different degree. Her figures, recalculated so as to yield b, are as follows:

Table XX

Muscle	b of chronaxie[1])
Gastrocnemius . .	0.61—0.86
Rectus anterior .	0.96
Sartorius	1.14—1.56

In general the "rapid" muscles show a lower coefficient than the "slow" ones.

According to F. B. Taylor and Alvarez (1917) there is a regular variation in the Q_{10} of the intestinal rhythm in the rabbit with the distance from the stomach. The variations are associated with the rapidity of the rhythm:

Table XXI

Segment	Contraction frequency at 38°	Q_{28-38}
Duodenum . . .	16.5	2.42
Jejunum	15.0	2.48
Upper ileum . .	13.0	2.88
Lower ileum . .	11.8	2.68

It remains to be investigated whether such differences in temperature coefficients are associated with the gradients of metabolism, detected by Child and his school.

14. Special case of mitosis

Different phases of the mitotic process are characterised by unlike temperature coefficients, as stated by Peter (1924) from the figures of Jolly (1903*a*, *b*) on the division rate of blood cells in the newt (*Triton*) and as confirmed by Ephrussi (1926, 1933) for the cleavage process in the sea-urchin and in *Ascaris*. Also Bucciante (1927*a*) has observed that temperature acted differ-

1) The same remark applies to this case as to the experiments of MacArthur and Baillie.

ently upon the anaphase and telophase in the mitosis of chick fibroblasts.

As regards analogous phenomena in plants, LAUGHLIN's (1919) figures on root cells of onion allow the conclusion that the Q_{10} here too varies during the mitotic division, being high in the interkinetic period, dropping in the prophase and rising again

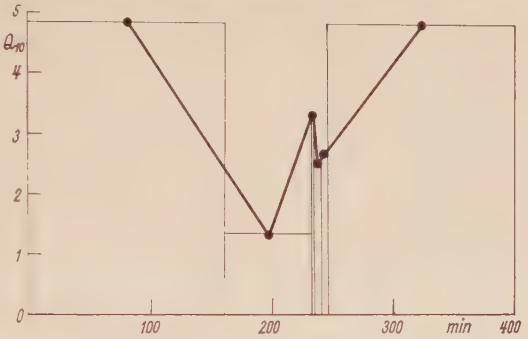


Fig. 25. Q_{10} of the duration of successive phases of mitosis in onion root-cells, according to LAUGHLIN. Abscissa: duration of the phase.

in the metaphase, anaphase and telophase (fig. 25). According to EPHRUSSI, the minimum value of Q_{10} coincides with the appearance of the equatorial plate.

15. Effect of thermal adaptation

BĚLEHRÁDEK (1926*a*, *b*) has pointed out that the temperature coefficients of analogous biological processes increase with the adaptation of the protoplasm to higher temperature, and later on (1931*a*) he illustrated this rule by numerous examples taken from the data of various investigators. ZAWADOWSKIJ and SIDOROV (1927, 1928) have confirmed this view in the cleavage process of eggs of different species and L. A. BROWN (1929*b*) in the rate of development in various related species of Cladocerans.

The examples given by BĚLEHRÁDEK concern germination of seeds, production of CO_2, velocity of amoeboid movement, activity of smooth muscles, heart beat frequency, cellular division, cleavage of eggs, embryonic development and locomotion speed in ants. For the detailed figures and for discussion it is necessary to consult the original paper. It may suffice here to reproduce some of the graphs (fig. 26—28) and to quote the case of developmental velocity in Cladocerans (L. A. BROWN 1929*b*):

Table XXII

Species	Temperature killing the animal within 1 minute	Temperature coefficient of development		
		Q_{10} (at 13°)	Q_{10}(at 25°)	b
Daphnia longispina . .	42°	1.82	1.48	1.00
Simocephalus .	43°	2.64	1.70	1.43
Daphnia pulex.	44°	2.36	1.81	1.63
Pseudosida bidentata . .	48°	2.90	2.03	1.80
Moina macrocopa . .	48°	3.96	2.39	2.15

Bělehrádek has suggested that temperature coefficients of vital activities must vary with thermal adjustment because of changes in the physical properties of protoplasmic fats and photophatids. This hypothesis will be discussed further on (IV. 9, *i*).

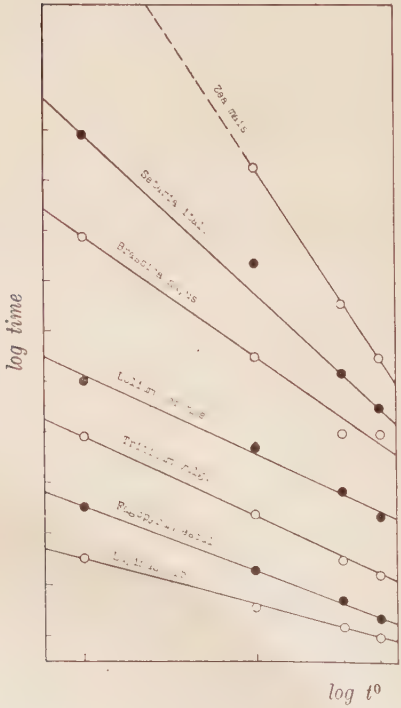


Fig. 26. Time required for germination of various seeds at different temperatures, in bilogarithmic representation (constructed by Bělehrádek 1931*a* from data of Haberlandt). The temperature coefficient b, is greater in thermophilic species, as is evident on the slope of the lines.

16. Effect of injury

After what has been said on the variability of temperature coefficients with many factors, it will not be surprising to see that injury, too, modifies the response of living systems to temperature.

Practically all the information available has been gained from the study of the

heart. SNYDER (1905) observed in the Pacific terrapin that the Q_{10} may either increase or decrease with advancing age of the heart preparation. FREUND (1922) found in the frog that the Q_{10} of heart beat frequency increases with the time elapsed after isolation, but GELLHORN (1924) observed a modification in the opposite direction.

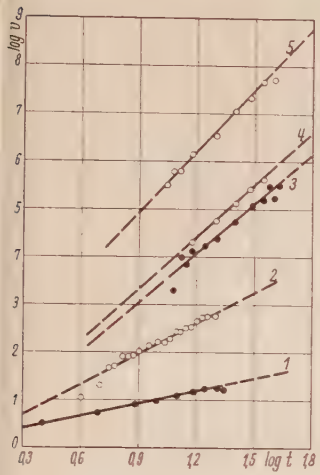


Fig. 27. Velocity (v) of ameboid movement in function with temperature (t^0). To avoid interference of the lines, the velocities extrapoled for $t^0 = +1^0$ C are put equal to unity. 1: marine amoebae (PANTIN 1924). 2: frog leucocytes (UEKI 1928). 3: rabbit leucocytes (SUGIYAMA and MORI 1927). 4: human leucocytes (SUGIYAMA and MORI 1927). 5: chick leucocytes (SUGIYAMA and MORI 1928). — The temperature coefficient, evident from the slope of the lines, is greater in homoiotherms than in poikilotherms.

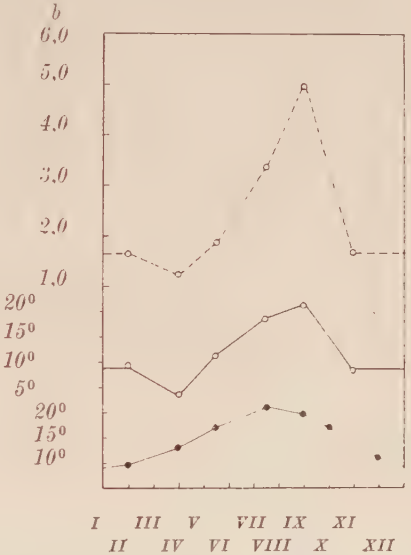


Fig. 28. Variation of the temperature coefficient b (above) and of the biological thermal zero (in the middle) of the locomotion of an ant, *Messor semirufus*, with the average monthly temperature (below) in Palestine. One division of the abscissa corresponds to one month, beginning with January. (Constructed by BĚLEHRÁDEK 1931*a* from data of BODENHEIMER and KLEIN.)

D. E. S. BROWN (1930*a*), working with strips cut out of the tortoise's auricle which he stimulated electrically, has seen a decrease in the value of μ with advancing age of the preparation:

Table XXIII

Age of strip	μ
0	14,800 ± 500
2 hours	14,000 ± 500
> 2 „	13,500 ± 500

Bělehrádek (1932*a*) has reported that the temperature coefficient of the rhythm in the isolated heart of the frog, perfused by a special method, remains constant for many hours and that even the changes in *b*, induced by caffeine, may be completely reversible. With guanidine however such reversibility is not possible (Bělehrádek and Mirski 1932).

Glaser (1925*a*) and Federighi (1929) have suggested that μ in cardiac activity might be modified by fatigue. E. and H. Gellhorn (1928) have found the following values of Q_{10} in the permeability of isolated frog skin, normal and injured, to dyes:

Table XXIV

	Q_{10} (between 15—25°) for	
	methylene blue	methylene azur II
Normal skin	2.13	2.38
Skin previously heated to 45°	3.34	3.39
Skin just before death	1.71	1.42

In this connection it may be noted that according to Osterhout (1914) the Q_{10} of the electric conductivity in *Laminaria* is smaller in dead than in fresh specimens (see further VII. 6).

Battelli and Stern (1913*a*, *b*) measured the O_2-consumption and CO_2-production at various temperatures in whole insects and in pulped insect tissue. Their results lead to the conclusion that the temperature coefficient is much lower in the second case:

Table XXV

	b	
	Intact	pulped
Fly larvae, O_2. .	1.50	0.48
„ „ CO_2 .	1.70	0.54
Cockchafer, O_2 .	1.72	0.58
„ CO_2.	1.80	0,73

CHAPTER IV

THEORIES OF TEMPERATURE COEFFICIENTS

1. Introductory and historical remarks

It is generally known that in the study of the kinetics of reactions between pure substances the notion of temperature coefficient played and still plays a dominant part. In reactions occurring between definite chemical bodies under determined conditions the temperature coefficient is found always to be of the same magnitude. This suggests the idea that the numerical value of the temperature coefficient is connected in one way or another with the intimate nature of the process in question. This idea became the starting point of much speculation in chemistry and gave rise to several theories, for details of which the reader is referred to special chemical treatises (Rice and Urey 1931 in H. S. Taylor 1931).

Some of these theories have been applied to the action of temperature upon the rate of biological activities in the hope that their intimate mechanism could thus be revealed. The purpose of the present chapter is to review these theories from a critical point of view along with some conclusions which have been drawn as to the kinetics of protoplasmic processes.

It will not be out of place to recall that the beginning of these speculations in biology was made by chemists and not by biologists. We have seen above (II. 4, II. 7) how chemists applied temperature formulae, which were first proposed for reactions between pure chemical substances, to vital phenomena. Theoretical implications of such formulae necessarily followed, and some of these have gained much popularity in biology, though they are considered as purely hypothetical even in chemistry. Such theories no doubt enable one to view the intimate kinetics of biological processes in a rather clear and simple way, but the

question arises whether such simplifications are fruitful in biology. It must not be overlooked that living systems differ greatly from the non-living ones, even from those of highest heterogeneity, by their complex character, caused by the presence of manifold phases, which themselves have a complex physico-chemical structure. In addition, different parts of living matter certainly offer very unequal resistance to the penetration of molecules, and various phase boundaries around and inside the cell may become important obstacles for the free diffusion of material, which may thus be prevented from reacting with the same velocity with which it would react in a non-living, homogeneous system.

Hitherto biologists have not imagined any theory of temperature coefficients of vital processes, and they have been content to borrow explanations from chemistry. As soon as a new hypothesis arose in chemical kinetics, it was tested with lesser or greater enthusiasm in biology. Yet biologists never have been fully satisfied with the explanations arrived at in this way and it will be shown in the subsequent paragraphs that they had many reasons for this scepticism.

2. Action of temperature upon "catenary series" of processes

There are relatively few instances among reactions between chemical bodies, which may be said to depend on a unique process. Such a case is realised in water transport between two solutions of different concentrations through a semi-permeable membrane, where the rate is dependent only upon the value of the osmotic pressure. When the action of temperature in such a system is investigated, it is found that the rate of transport increases in the same measure as does the osmotic pressure.

When a crystal is dissolved in water, the velocity of dissolution depends upon the velocities of two different processes, *i. e.* upon the rate at which free molecules are liberated at the interface between the crystal and water, and upon the rapidity with which the liberated molecules will move away from this interface. The reaction as a whole is thus based upon a "catenary series" of successive reactions. When the system is heated, it may be found that the rate of the whole process is increased and a temperature coefficient may be calculated. Now it is obvious that

temperature might have affected either of the two partial processes in a different way and the question arises in which way. In this special case, the system may be stirred or agitated so that the diffusion shells around the crystal are steadily destroyed and free molucules now move away at a speed much greater than in the first case, and independently of temperature. If the experiment, thus modified, reveals a temperature coefficient identical in value with that previously found, it is to be concluded that in both experiments we are faced with the phenomenon of temperature action upon the rate of liberation of molecules. If, on the contrary, the temperature coefficients are unlike, it will be obvious that we have a temperature coefficient of diffusion velocity in the first case and a temperature coefficient of liberation of molecules in the second.

It may be safely concluded that vital phenomena are also based upon catenary series of reactions, which, however, are more complicated than that just discussed. Even when dealing with very simple phenomena, such as for instance haemolysis by hypotonic solutions, at least two separate factors must be taken into account, the rate of water permeation, and the mechanical properties of the cellular surface, which, too, is modified by temperature. Yet in the greater majority of cases the vital phenomena are based upon still more complex chains of reactions, some of which certainly are "physical" in nature, involving especially the transport of the material from without to the cell surface, through this surface, through cytoplasm, and the absorption processes, the other reactions involved being of a chemical character: chemical combination, substitution, splitting, oxidation, reduction, etc.

Let us consider more closely an ideal case of a catenary series composed of two processes occurring at velocities D and K respectively, whilst the velocity of the whole reaction is W. Say that D is a diffusion process, which yields material for a combining reaction K. Let us suppose further that temperature accelerates both of these processes in unlike degrees, the first being associated with a $Q_{10} = 1.1$, the other with a $Q_{10} = 2$. Now two different cases will present themselves according to the relative velocities of the two processes. Supposing that the diffusion is rapid ($D = 10$ at 0^0) and the combining process slow ($K = 2$), the resulting Q_{10} of the whole catenary series will be as follows:

Table XXVI

t^0	D	K	W	Q_{10}
0^0	10	2	2	2.0
10^0	11	4	4	2.0
20^0	12.1	8	8	1.7
30^0	13.3	(16)	13.3	1.1
40^0	14.6	(32)	14.6	1.1
50^0	16.1	(64)	16.1	

In this case, from 30^0 onwards D cannot keep pace with K, and K therefore is limited by D, on which it depends. There will be found a double value of Q_{10} with a break at $20—30^0$, which is a "critical" temperature, at which the process D becomes the "limiting factor" or "master process" of the whole. Yet it may occur that on the contrary D is slow in relation to K. Say that K again is 2, but D is 1. In this case the resulting value of Q_{10} will be:

Table XXVII

t^0	D	K	W	Q_{10}
0^0	1	(2)	1	1.1
10^0	1.1	(4)	1.1	1.1
20^0	1.21	(8)	1.21	1.1
30^0	1.33	(16)	1.33	1.1
40^0	1.46	(32)	1.46	1.1
50^0	1.61	(64)	1.61	

Here then a single value for Q_{10} will be found throughout the whole temperature range, equal to the value associated with the process D.

This scheme to which of course could be applied any of the temperature coefficients discussed in Chapter II, provided they are constant for the basic processes in consideration, is of much importance for the understanding of the principal theories of temperature action in biology. It was proposed in its original form by Blackman (1905), J. Loeb (1912), Pütter (1914) and Osterhout (1917*a*). Limitations of its use have been discussed by Bliss (1926), its application to inorganic chemistry especially by Hinshelwood (1926). (See also Glaser 1924.)

3. Classification of biological processes into "physical" and "chemical"

We have just seen (IV. 2) that vital phenomena may be conceived as being based upon a "catenary series" of reactions. In any such series the actual velocity of the whole is determined by the velocity of its slowest member. It would thus be possible to compare the values of temperature coefficients in various vital activities and to identify the intimate biochemical basis of different processes.

The first to attempt to draw such conclusions in biology was Vernon (1895*a*), who found long before the Q_{10}-rule came into common practice, that temperature affected pulsation frequency and oxygen consumption in Medusa in different ways. The idea gained ground especially when Snyder (1905, 1907*a*, 1908*a*. *b*. 1911) argued that the heart beat was chemical in nature because it gave a high Q_{10}, while the conduction of nervous impulse was of a physical nature because it yielded a low Q_{10}. These conclusions were based on the generally admitted assumption that the diffusion process and other physical phenomena depend but little on temperature and thus give low values of Q_{10}, while chemical reactions give a Q_{10} greater than two. Snyder's (1908*b*) study also brought the rather unexpected result that water permeation through frog skin is connected with a $Q_{10} = 2.0—2.5$. Hence the conclusion that water resorption is due to a chemical and not to a physical mechanism. Snyder (1908, 1911) also expressed the idea that in cases where unlike values of Q_{10} are found at different temperatures, the reaction is based upon several distinct processes.

These views gained much popularity in the subsequent years and many workers investigated temperature coefficients with the view to obtain information as to which processes are based upon a physical, and which upon a chemical mechanism. This work is connected with the names of Maxwell (1907), K. Lucas (1908—1909), Robertson (1908), Sutherland (1908), J. Loeb (1908*a*, 1912), Moore (1910*b*), Rutgers (1911), J. Loeb and Wasteneys (1911), Piper (1912), Warburg (1914), Pütter (1914), Osterhout (1914, 1917*a*), Adrian (1916), Serebrovskij (1916), Broemser (1921), Garrey (1921) and many others.

In connection with this work it was often found that processes which might have been conceived *a priori* as being of a

physical nature, yielded high values of Q_{10}, and it became usual to conclude that they are in reality based on a chemical reaction. Besides the conclusion of SNYDER quoted above, that the resorption of water by frog skin has a chemical basis, similar views were accepted by W. STILES and JÖRGENSEN (1915) for the permeation of HCl into plant cells, by CROZIER (1922) for the permeation of the same acid into certain animal cells, by M. IRWIN (1925) for vital staining, by E. and H. GELLHORN (1926) for the transport of certain dyes through animal membranes, and by HOAGLAND, HIBBARD and DAVIS (1926) for the accumulation of Br in *Nitella*.

Yet such conclusions, though repeatedly arrived at by various authors, are rather problematic. As a matter of fact BOYCOTT as early as 1902 claimed that it is not possible to recognize the nature of the process from its mere response to temperature, and similarly K. LUCAS (1908) has pointed out that a high value of Q_{10} does not with certainty exclude physical mechanism. Also KANITZ (1915) has laid stress upon the fact that some physical phenomena, for instance the viscosity of very viscous liquids, give high values of Q_{10}. There is now plentiful evidence on hand that many phenomena, even when certainly based upon a physical mechanism, have a high temperature coefficient. This point will be discussed in full later (IV. 7). It may suffice here to state that the discrimination between "physical" and "chemical" processes in biology by means of temperature coefficients has no real basis and that it should be abandoned.

4. Identification of vital processes and of their biochemical basis

When one and the same biological process gives two or more different temperature coefficients under various conditions, three different explanations are possible: either that the formula used does not adequately express the relation between temperature and velocity in that particular case, and another type of formula should be tested; or, if the formula holds good, that the value of the temperature coefficient is modified by the conditions at which the reaction takes place; or, finally, that there are two or more distinct reactions on which the whole process is based under varying conditions.

From what has been said in the two preceding chapters it is clear that the last of these explanations is the least probable and that the first two possibilities should be excluded before recurring to the third one. Yet it was and still is a common practice in many investigations to admit the last of the hypotheses without testing the first two.

Thus when it is found that Q_{10} in respiratory movements is unequal in abundance and in lack of O_2, it is not necessary to conclude that there are two nervous mechanisms in play, the one for low and the other for high O_2 pressures, but it may be that there is a single mechanism, based upon a definite series of protoplasmic reactions, whose Q_{10} is modified according to the amount of oxygen at hand (Babák and Roček 1909; see also III.6.) Similarly, when various Q_{10}-values for heart beat frequency are established in different stages of development, it is not admissible to suppose that the heart beat is governed by several various sets of reactions at different stages, before it is shown that the variations of Q_{10} with age are not systematic and continuous, but abrupt (see Cesana 1912, Garrey 1920, Crozier and Stier 1927*b*, Parpart and Glaser 1930). — The same remark applies as well to some recent explanations of individual variability of temperature coefficients (Federighi 1930).

The idea that it would be possible to reveal the biochemical mechanisms of vital processes by means of their temperature coefficients is to be ranged into one and the same line of thought with the concept that it would be possible to reveal by the same method whether a process is "chemical" or "physical" in nature (see IV.3). In studying the literature from the first fifteen years of the present century one may easily trace the genetical relationship between these two principles, the first of which evidently is only a less daring expression of the other. Among the protagonists of the hypothesis that the numerical value of Q_{10} is connected with the biochemical nature of vital activities were Snyder, J. Loeb, Lucas, Moore, Babák an Roček, Cesana, Adrian and many others. The appearance of the majority of these papers coincides with the years during which the use of Q_{10} and its theoretical implications were very popular. Yet the idea has not been forgotten since and has been developed of late with emphasis by Crozier and his adherents in connection with the use of the Arrhenius formula (see especially: Crozier 1924—1926, Crozier

and collaborators 1924—1931, Bliss 1926, L. A. Brown 1926, Castle 1928, Cole 1925—1926, Cole and Allison 1929, Federighi 1929–30, Fries 1926, Glaser 1924—29, Hecht 1926—28, Navez 1928—1931, Orr 1925, Parpart and Glaser 1930, Pincus 1931, Richards 1928, Snyder 1931, Steiner 1932, Stier 1933, Stier and Pincus 1928, Stier and Wolf 1932, Tang 1932*a*, *b*, Wolf 1932, etc).

To this renewed idea, however, Crozier made an important addition. First, he systematically uses the Arrhenius formula (II.7), which he believes to agree more closely with the observed data than does the Q_{10}-rule. Second, he applies the theory of this formula, according to which the constant μ denotes the heat of activation involved in the given reaction and characteristic for a definite catalyst, to biological phenomena, and he thinks that various processes, in which the same or a similar value of μ is found, may be conceived of as being based upon one and the same biochemical mechanism. So he concluded (1924*c*) that processes associated with $\mu = 16{,}700$ are based upon oxidations produced by iron dehydrogenation, while those yielding a $\mu = 11{,}500$ are based upon oxidations induced by OH', and those in which $\mu = 20{,}000$ upon oxidations by means of H·ions.

There is still a third point in Crozier's hypothesis, *i.e.* that for one and the same process various magnitudes of μ are often found for different temperature intervals. The value of μ is thus found to change abruptly at a definite temperature, which is "critical". This is explained by Crozier by the assumption that in the "catenary series" of reactions (see IV. 2) underlying the process in question, different members may become slowest at different temperatures, and that they thus limit the velocity ofthe whole chain.

Against the views of Crozier and of his followers some authors have offered criticisms (Heilbrunn 1925, Bělehrádek 1928*b*, 1929, 1930*a*, H. W. Harvey 1928, Emerson 1929, Fulmer and Buchanan 1929, D. E. S. Brown 1930*a*, Gerhart 1930, Haldane 1930, Kurbatov and Leonov 1930, Ponder and Yeager 1930, Yamamoto 1931). The answers of Crozier and of his pupils, which must be read in the original papers (Crozier and Navez 1931; Navez 1931; see further Bělehrádek 1932*b*), naturally tend to support the original views, but there still remain some facts which make Crozier's hypothesis extremely doubtful. The fundamental objections are summed up as follows:

(*a*) As to the assumed advantages of the ARRHENIUS' formula, we have seen above (II.7) that these are non-existent, because for the short range of biokinetic temperatures this formula may be simply reduced to the Q_{10}-rule.

(*b*) It is true that in modern chemistry there is a tendency to attribute to the constant μ the significance of the heat of activation, which is different for different reactions and which also varies with the nature of the catalyst, but it must be kept in mind that this way of facing the problem is only one of several admissible hypotheses now discussed in chemistry (see especially RICE and UREY 1931). It would certainly be premature to introduce any of these theories into biology until the problem is sufficiently cleared up in chemistry.

(*c*) Even if this theory proves correct in chemistry, it could not yet be applied to living activities, because the value of μ certainly varies with many internal and external factors, the chief of which have been enumerated in the preceding chapter. One can scarcely hope, therefore, that the μ of one and the same protoplasmic activity would be constant enough to allow a comparison and a classification of processes. Even CROZIER and his adherents themselves (CROZIER 1926*a*, *b*, CROZIER and STIER 1926*a*: GLASER 1929) are obliged to admit that the values of μ may be modified for chemical reasons, that they greatly vary in different individuals of the same species and that they also can be "distorted" by purely physical agents (GLASER). The question however arises as to how important these "distortions" actually are and whether they attain such a degree that the real values of μ would be entirely veiled. As far as our present knowledge reaches, such a state of affairs is to be expected.

(*d*) The fact that different values of μ are often found for various temperature intervals which are limited by "critical temperatures", is clearly connected with what is known to occur with the Q_{10}. There are no sudden "breaks" in the temperature-velocity curves, be they constructed by the method of μ or by that of Q_{10}, but these values vary with temperature in a progressive and systematic way not only in enzyme action, but also in protoplasmic processes (see II.5, 7). The frequency of "critical temperatures" alone is so great that practically any temperature may be conceived of as critical in this sense. CROZIER (1926*b*) quotes the following as the most frequent "critical temperatures":

4.5°, 9°, 15°, 20°, 25°, 27°, 30°. The straight lines, which in CROZIER's representation yield various values of μ, are simply tangents to a more complex curve, which are drawn more or less arbitrarily. This can best be illustrated by the fact that a single temperature coefficient properly chosen suffices to describe the relation where two or more values of μ are found, as shown by Figs. 11—12 (see p. 22). Likewise the results of E. G. MARTIN (1904), from which CROZIER (1924*c*) calculates two different values of μ, are represented with sufficient accuracy by means of a single value of b (see fig. 16, p. 28).

It will be interesting to recall that a similar controversy arose between SNYDER and E. N. HARVEY (1911) as to the occurrence of "breaks" in Q_{10} curves. HARVEY argued that Q_{10} cannot be used for a classification of the reactions, which are at the basis of vital activities, because similar variations of Q_{10} with temperature, which are known also in enzymes, are continuous and not sudden. The same remark must likewise be applied nowadays to the work of CROZIER and of his collaborators.

(*e*) The only argument which might be quoted in favor of these views, is the numerical identity of temperature coefficients in many biological activities with those in many chemical reactions. But this identity is often very doubtful, because Q_{10} and μ vary with the temperature chosen, and even if it were not so, there is a possibility that the same value of temperature coefficient is associated for example with phenomena of diffusion.

5. Photochemical reactions in biology

It is generally believed that photochemical reactions are independent of temperature, giving thus a $Q_{10} = 1$ or slightly over 1, as first suggested by GOLDBERG (1903). But it has been shown by PADOA and MINGANTI (1915) that the Q_{10} changes in value with the wave-length of the absorbed light, and that it may even attain a value of 1.50—1.75, especially in the presence of sensitisers.

It would be out of place to discuss here the general theory of photochemical processes in chemistry. A very intelligible review of the current views may be found in H. S. TAYLOR's treatise (1931).

The most important point in these theories undoubtedly is, as regards the biological temperature problem, that the magnitude

of Q_{10} should increase with decreasing wave-length (λ). This is postulated by the formula of PERRIN:

$$\lambda = \varrho \frac{6{,}2}{log\, Q_{10} \cdot T_1 (T_1 + 10)} \qquad \text{(XXVIII)}$$

in which ϱ is a constant and T the temperature. The following table contains Q_{10} values of various photochemical reactions in biology:

Table XXVIII

Reaction	Author	Q_{10}
(*a*) *visible light*:		
Bleching of retinal pigment . . .	S. HECHT (1920)	1.0
Threshold of visual process in *Mya arenaria*	S. HECHT (1920)	1.05
Photolysis of luminescent granules in *Eucharis*	A. R. MOORE (1926)	1.1—1.3
(*b*) *ultraviolet rays*:		
Stimulation of *Cyclops*	V. HENRI and V. HENRI (1912)	~1.0
Killing of yeast cells	LÜERS and CHRISTOPH (1923)	1.17
Killing of infusoria	L. HILL and EIDENOW (1924)	1.20—1.60
Browning of leaves	M. T. MARTIN and WESTBROOK (1930)	~1.0
Killing of Staphylococcus	GATES (1929)	1.10
(*c*) *x-rays*:		
Destruction of eggs of *Ascaris* (0,7Å)	DOGNON (1925)	1.50
Destruction of eggs of *Ascaris* (0,4Å)	DOGNON (1925)	1.61
Destruction of eggs of *Ascaris* . . .	HOLTHUSEN (1921)	1.5—2.3
Destruction of chick embryo . . .	ANCEL and WINTERBERGER (1925)	about 1.0

Though there seems to be a general trend in these figures to follow the theoretic rule, it cannot be said that PERRIN's formula is fully satisfied. In addition, there are many processes known in biology in which a high Q_{10} value is found in spite of the rôle played by light in them, for instance in photosynthesis (see OSTERHOUT and HAAS 1918), phototropic responses, appearance

of skin erythema on irradiation by ultraviolet rays (L. Hill and Eidenow 1924) and many others. In such cases it is generally admitted that the process again is based upon a catenary series of reactions, the photochemical reaction being one of them, and

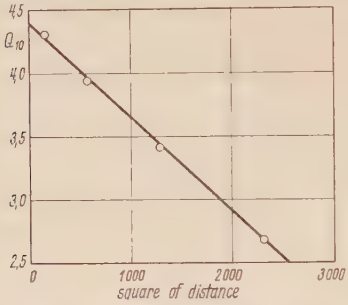


Fig. 29. Q_{10} of the duration of mortal exposure to ultraviolet light of infusoria decreases in value with the intensity of radiation. (Calculated from results of Hill and Eidenow.)

that the rate of the whole is limited by another "master process", which is not photochemical in nature (Osterhout and Haas; Emerson and Arnold 1932; S. Hecht 1920, 1926).

It is interesting to note that, from the experiments of L. Hill and Eidenow on the rate of killing of Infusoria by ultraviolet light, a definite relation between Q_{10} and intensity may be derived (see fig. 29):

Table XXIX

Distance (inches)	Relative intensity	$Q_{11-20°}$
12	694	3.84
24	174	3.55
36	77	3.09
48	43	2.48

One of the most recent hypotheses on the kinetics of chemical reactions is that maintained by Trautz and Perrin (see Rice and Urey 1931) according to which the ordinary or "dark" reactions are produced by infra-red rays, which activate the molecules. This view has not yet been sufficiently tested in chemistry, but in spite of it some workers have already attemped to introduce it into biology (Baas Becking 1921; Snyder 1931). Baas Becking thinks that there is a close agreement between the values of Q_{10} predicted by this theory and those found experimentally in biology, but to obtain such agreement it was necessary

to modify the magnitude of ϱ in PERRIN's equation given above (XXVIII). Also SNYDER, who calculated on the same basis the number of quanta involved in biological "dark" reactions, finds that the experiments agree fairly well with the theoretic values.

However interesting such attempts appear, they certainly are at least premature in regard not only to the present state of our biological knowledge, but also to the state of contemporary discussions in chemical kinetics. Besides, it is highly questionable whether such constants as Q_{10} and μ, which in biological "dark" reactions greatly vary with temperature, could ever become the basis of a serious comparison for similar purposes.

Values of Q_{10} for photochemical reactions in biology have been tabulated recently by KANITZ (1925) and by KRÜGER (1931).

6. SKRABAL's hypothesis

SKRABAL (1916) has established an empirical rule according to which a close relation exists between the reaction velocity and its temperature coefficient. Chemical reactions, which proceed at high velocity, have low temperature coefficients, whilst in slow reactions temperature coefficients are high. SKRABAL also has shown that when a reaction is slowed by a negative catalyst, its temperature coefficient rises, and *vice versa*. Also photochemical reactions follow this general rule, as they are almost independent of temperature because of their great velocity. When the same reaction occurs slowly without light absorption ("dark" reaction), the temperature coefficient accordingly is high. For further examples and for a more detailed discussion of the theory, the original paper of SKRABAL must be consulted.

SKRABAL at the same time has given some examples to show that the rule applies as well to biological phenomena. He has pointed out that rapid processes, *e. g.*, nervous conduction, yield low temperature coefficients, whereas in growth, development and in other slow phenomena the temperature coefficients are high. F. and G. WEBER (1916) have demonstrated that this rule applies to the viscosity of protoplasm as modified by temperature. In highly viscous protoplasms the effect of temperature is more important than in liquid ones.

Though the relation between the importance of temperature action and the velocity of vital processes was discussed as early as 1872 by ROSSBACH and later by SEREBROVSKIJ (1916),

nobody has as yet attempted to test the validity of SKRABAL's views on a more satisfactory number of biological processes.

It is not difficult to collect numerous data to test the hypothesis. We have seen above (III. 13) that muscles which show a "rapid" excitability yield lower values of b than those which may be called "slow", according to FILON. In the same connection the experiments of TAYLOR and ALVAREZ have been quoted, which, too, seem to support SKRABAL's idea. ZAVADOVSKIJ and SIDOROV (1927), apparently without knowing SKRABAL's work, give the following figures which are in agreement with the theory:

Table XXX

Species	Strongylocentrotus purpuratus	Rana buty rrhina	Sphaerechinus granularis	Echinus microtuberculatus	Arbacia sp.	Ascaris megalocephala
Rate of cleavage .	rapid	>	>	>	>	slow
Q_{10} of cleavage. .	1.82	2.1	2.4	3.2	3.6	>5

Undoubtedly many other similar instances could be quoted from the biological results now at hand, but it is more important to show that there are likewise numerous cases in which the data do not agree with the theory. Table XXXI contains some figures on heart beat frequency in diverse animals, tabulated in increasing order of frequency, which clearly show that there is no absolute relationship between the rate and its temperature coefficient.

Table XXXI

Species	Heart-beat frequency per 1 min. at 20°	b	Authority
Limulus polyphemus	0.37	1.46	GARREY 1920
Terrapene clausa, 0.11 % KCl .	2	4.70	E. G. MARTIN 1904
Anodonta	2.3	1.18	KOCH 1917
Terrapene clausa, without KCl .	14	0.84	E. G. MARTIN 1904
Mya arenaria	15	1.64	E. YUNG 1888
Nereis virens	17	1.60	ROGERS 1911
Terrapene clausa, 0.08 % KCl .	17	2.80	E. G. MARTIN 1904
Duck embryos	20	2.92	INUKAI 1925
Helix pomatia, winter	26	1.28	YUNG 1888
Terrapene clausa, normal . . .	28	3.14	E. G. MARTIN 1904
Lacerta agilis, embryo 5 mm. .	28.3	1.88	ANDERSEN 1929a

Species	Heart-beat frequency per 1 min. at 20°	b	Authority
Lymnaea auriculata, adult . . .	29.6	1.76	Richard 1888
Xenopus laevis	31.6	1.54	A. J. Clark 1921
Hynobius lichenatus, larva . . .	33	1.15	Inukai 1925
Clemmys marmorata	34.5	1.42	Snyder 1911
Rana temporaria	37.5	1.04	A. J. Clark 1921
Rana temporaria	40	1.19	A. J. Clark 1921
Lymnaea auriculata, young . .	40	1.76	Richard 1888
Lacerta agilis, embryos 10 mm .	42.5	2.02	Andersen 1929a
Rabbit	43.5	1.96	A. J. Clark 1921
Phyllirrhoe	46.5	1.28	Snyder 1907b
Helix pomatia	50	1.00	Richard 1888
Cat	51	2.25	Britton 1922
Lacerta agilis, embryos 11.5 mm	56.3	2.40	Andersen 1929a
Frog tadpoles	68	1.05	Bataillon 1891
Helix pomatia, summer . . .	87	1.19	Yung 1888
Fundulus heteroclitus, embryos .	93.7	1.58	Glaser 1929
Fundulus heteroclitus, embryos .	97.4	1.48	Loeb and Ewald 1913
Trutta fario, embryos 10 mm .	108	1.05	Andersen 1929b
Trutta fario, embryos	120	1.06	Babák 1912
Trutta fario, embryos 20 mm .	142	1.19	Andersen 1929b
Ceriodaphnia	180	1.14	Robertson 1906
Daphnia pulex	214	1.00	Bělehrádek 1929
Daphnia magna, female . . .	256	1.10	MacArthur and Baillie 1929b
Daphnia magna, male	330	1.26	MacArthur and Baillie 1929b

The empirical rule suggested by Skrabal therefore cannot be said to hold good in biological processes, except in a very general way.

7. Possible rôle of diffusion and of viscosity

In the kinetics of chemical reactions two different cases must be distinguished, *viz.* reactions in homogeneous and in heterogeneous systems. A heterogeneous system is characterised by the presence of phases, which are separated by interfaces. Living matter is a heterogeneous system, and the laws which have been established for such systems necessarily aply to it.

In heterogeneous systems reactions take place at the interfaces. Their net velocity will depend not only on the rate at which the chemical change itself occurs at the interface, but also on the rate at which the necessary material is carried to the interface, and on that at which the products are removed from it. The fundamental notion of this relation is due to NERNST (1904) and to BRUNNER (1904). Any reaction of this sort may be, in the simplest case, represented by the following scheme:

$$\xrightarrow{D_1} \quad \xrightarrow{K} \quad \xrightarrow{D_2}$$

in which D_1 and D_2 denote the diffusion process to and from the interface respectively, and K the chemical process itself.

From what has been said with regard to the catenary series of reactions (see IV.2), it is clear that the net velocity of the whole chain depends on the rate of the slowest stage. It may be that diffusion is the slowest process, but it may also be that it is quicker than the reaction itself. In the chemistry of pure substances it is often possible to distinguish between the two cases by investigating whether stirring has or has not an effect upon the rate. It is known that the velocity of diffusion is proportional to the 2/3 or 4/5 power of the velocity of stirring (BRUNNER 1904; H. S. TAYLOR 1931). Thus when it is found that stirring increases the velocity of the process, it may be concluded that the latter is governed by the rate of diffusion (see IV.2). In protoplasmic reactions such a test generally cannot be undertaken, yet there are some experiments now at hand which show that some processes are in fact dependent on the rate of protoplasmic streaming (BIERBERG 1909; yet compare KOK 1931) or on the rate of stirring the medium (PFEFFER 1886, STEWARD 1932). There are, further, many mechanisms in living bodies, which accelerate the diffusion of reacting substances by streaming, stirring or agitation (respiratory movements, peristaltic movements, circulation of blood, etc.), thus suggesting that the velocity of the respective processes is limited by too slow a diffusion.

The viscosity of the protoplasm and of its constituents gives special reason to suspect that the diffusion might be the slowest process in protoplasmic chains of reactions. As a matter of fact this was often admitted even in the earliest investigations and in spite of a complete lack of satisfactory evidence the idea never was

entirely abandoned (Blackman 1905, Balls 1908, Sutherland 1908, Snyder 1909, 1911, Cohen Stuart 1911, Rutgers 1911, van Amstel and Iterson 1911*b*, Pütter 1911, 1914, Czapek 1913, Masing 1914, Warburg 1914, Traube 1914, Serebrovskij 1916, F. and G. Weber 1916, Osterhout 1917*a*, Bayliss 1918, Dhar 1920, Fenn 1922*a*, Przibram 1923, Warburg 1923, Jacobs 1924, Pantin 1924, Fauré-Fremiet 1924, Heilbrunn 1925, A. V. Hill 1928, K. Meyer 1930, etc.). — For the special case of permeability as dependent on protoplasmic viscosity, see Krabbe 1896, Spaeth 1916, Osterhout 1916, Stiles 1924, Höfler and Weber 1926, Huber and Höfler 1930. More recently Bělehrádek (1926*c* — 1932*b*) has attempted to show that there is now much evidence in favor of such views. He juxtaposes the "chemical" theory of temperature coefficients to the "physical" one and thinks that the velocity of protoplasmic processes is always or nearly always limited by the low diffusion velocity in viscous parts of the protoplasm.

Before entering into the discussion of this possibility, it will be necessary to consider more closely the viscosity, the diffusion and the reaction velocity in their mutual relations.

The relation between viscosity and diffusion is described by the well-known formula of Einstein and Smoluchowski:

$$D = \frac{k \cdot RT}{N} \cdot \frac{1}{6\pi\,\eta\,r} \qquad \text{(XXIX)}$$

in which D is the diffusion coefficient, R the gas constant, N the Avogadro's number, T the absolute temperature, η the viscosity and r the radius of the diffusing molecule. This formula has often been found to be sufficiently accurate and the rate of diffusion may be considered as being inversely proportional to the viscosity. For some special cases, however, corrections appear unavoidable, especially for the diffusion of electrolytes in water and in watery media (Pissarjewski and Karp 1908; see also Hatschek 1928). Stiles (1930*b*) has objected that this formula cannot be used as a basis of theoretical speculation in biology, because it does not embrace with uniform accuracy all the diffusion phenomena observed. It must however be borne in mind that in spite of such irregularities the general conclusion still holds good that diffusion does depend on viscosity, even when more complicately than is postulated by the formula (see Bělehrádek 1932*b*).

There is only one evidence of this relation as far as protoplasm is concerned, namely the results of DIETRICH (1929), showing that protoplasmic viscosity, evaluated by means of the Brownian movement, determines the rate of diffusion of chrysoidine in fungal filaments.

As to the relation with the reaction velocity itself, there is now available in chemistry extensive indication that viscosity affects the net velocity, whenever the diffusion process is slower than the reaction (BUCHBÖCK 1900, NERNST 1904, BRUNNER 1904, SCHILOW and PUDOFKIN 1910, GRUMELL 1911; see also the reviews of KREMANN 1928, p. 271 and of H. S. TAYLOR 1931, p. 1019). Also in enzyme action studied *in vitro* the rate is dependent on the viscosity, at least in some cases, whilst in other cases no such relation has been detected. It is probable that under different experimental conditions diffusion may or may not become the slowest "link" (see especially HERZOG 1904, ACHALME and BRESSON 1911, COLIN and CHAUDUN 1922, 1929, INGERSOLL 1926, ACHALME 1926; compare also KUHN 1924, p. 260 seq.).

There is however an apparent flaw in the idea that the temperature coefficients of biological reactions would be identical with those of the rate of diffusion in protoplasm, namely the low numerical value of temperature coefficients of viscosity and of diffusion as compared to those of most biological processes. For this reason SNYDER (1909) refused to accept SUTHERLAND's views on the physical nature of the conduction of nervous impulse and together with TODD (SNYDER and TODD 1911) he studied the viscosity of various organic liquids from this point of view. The viscosity of cytoplasm, measured by various methods, too, yields in the majority of cases Q_{10} values much lower than is customary in biological reactions themselves (see VII.1).

This objection however is not sufficiently founded in the light of information now at hand. F. and G. WEBER (1916) have pointed out that when a process depends on diffusion, which itself is associated with a $Q_{10} = 1.2$, the resulting Q_{10} of the whole process will be equal to $1.2^2 = 2.07$, as is postulated by PÜTTER's principle. Furthermore it must not be forgotten that what we are measuring by the direct viscosimetry in protoplasm concerns the most liquid phase of living matter, and that probably the viscosity of other protoplasmic phases of higher consistency would be dependent on temperature to a more important degree, as is the case in highly

viscous bodies in general (see the next paragraph). This applies in the first place to the regions adjacent to phase boundaries, where the cytoplasm may become highly condensed. It was first pointed out by KANITZ (1915) that some cases of diffusion and of viscosity are characterised by a high Q_{10}. As a matter of fact, the notion of low Q_{10} values of diffusion velocity is almost entirely derived from the study of electrolytes and other simple substances diffusing into water or into jellies of high water content. But DAVIS and JONES (1912) have found that the electric conductivity of some salts in glycerine yields $Q_{10} = 2$ (between 25° and 35°). As found by GULDBERG and WAAGE, the rate of dissolution of a metal in concentrated HCl, too, is accompanied by a high temperature coefficient (p. 68). Thus a high Q_{10} does not ensure that the underlying process is not diffusion — a conclusion reached even by some earlier investigators, quoted above (p. 50).

Many recent workers are now inclined to admit that biological processes which are associated with a high temperature coefficient still may be based upon diffusion. M. IRWIN (1926), speculating as to the nature of the *intra-vitam* staining, claims that a high Q_{10}, which she found in some cases, does not exclude diffusion as the underlying process. The most striking confirmation however is that furnished by MCCUTCHEON and LUCKÉ (1926, 1927). These investigators studied the kinetics of water endosmosis and exomosis in the eggs of *Arbacia punctulata* and have stated that the process gives a $Q_{10} = 2$ to 3, or a $\mu = 13{,}000$ to 19,000, in spite of its being clearly dependent on diffusion, as shown by the study of the volume change in time. Evidently all the earlier conclusions in which a "chemical nature" was supposed for phenomena of permeability when a high Q_{10} was found, must be altered (see also STILES 1924, p. 147, 206 seq.).

But there still remains another obstacle, *i. e.* that some direct evaluations of protoplasmic viscosity as a function of temperature reveal a more complex interrelation with a marked maximum of viscosity in the neighbourhood of 15—16°, beside the habitual maxima at very low and high temperatures. These findings will be discussed further on (VII. 1) along with some objections (see also BĚLEHRÁDEK 1932*b*). Suffice it to say here that they cannot be regarded as being of a general significance for any kind of cytoplasm. Besides, it is to be noted that they again concern only

the most liquid phase of living matter and that viscosity, taken as obstacle of free flow, may essentially differ from viscosity taken as obstacle of free diffusion (see VII.1). Furthermore in such phenomena, which almost certainly are intimately connected with cytoplasmic viscosity, as for instance in amoeboid movement and in protoplasmic streaming, no maximum at 15—16° can be revealed (see Heilbronn 1922, Pantin 1924). Heilbrunn (1928) quotes from Crozier (1924c) that in the vicinity of 15° some deviations occur from the theoretical temperature-velocity curve in Arrhenius' representation, and he thinks that they should be attributed to the maximum of viscosity found at that temperature. But Crozier has given many other similar "critical" temperatures (see p. 53—54) and one might similarly suppose that any of them is based upon an analogous maximum. There ought thus to be revealed as many maxima of viscosity in protoplasm as "critical" temperatures found in a given process. Besides, we have seen that the notion of "critical temperatures" is highly questionable (IV.4*d*). The W-shaped curve of Heilbrunn and others cannot therefore furnish any obstacle to the view that the viscosity of various protoplasmic phases greatly retards the diffusion and that the latter may often be the "master process" of the chain of biochemical reactions.

8. Time-factor in the action of temperature upon the viscosity and upon the rate of biological processes

As there is no possibility of measuring directly either the rate at which various substances diffuse to and from the protoplasmic interfaces, or the real velocity at which biochemical processes do proceed at interfaces, both hypotheses, the "chemical" and the "physical", appear equally justified. In principle the "chemical" hypothesis supposes that the diffusion is more rapid than the reaction itself, whereas the "physical" one supposes on the contrary that the diffusion is slower. Though many experimental data on the transport velocity of various substances into and from the cells show that the diffusion often is very slow in protoplasm (see examples in Bělehrádek 1932*b*), there is no direct way of testing the two opinions, unless the rate of biochemical reactions at the interfaces can be determined.

There are however two indirect methods of testing the validity of the "physical" view, as suggested by Bělehrádek (1926*b*, 1932*b*; see also Bělehrádek and Mirski 1932).

The first method is based upon the study of the time-factor in the action of temperature in biology. It is generally known that the viscosity of hydrophile colloids at a given temperature changes a long time after the temperature equilibrium has been reached. This property of colloids was studied in particular by J. Loeb (1922), although it was known before. Nobody as yet has attempted to examine systematically the protoplasmic viscosity from this standpoint (see Weber and Hohenegger 1923). But if it is true that the velocity of vital processes is governed by the rate of diffusion in a viscous collodial medium, it must

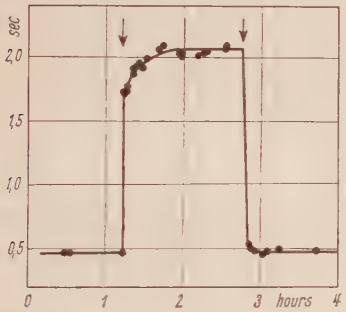


Fig. 30. Time-factor in the action of cold upon the frequency of the heart in larval trout. Abscissa: duration of the experiment, in hours. Ordinate: time required for one heart revolution, in seconds. Arrows to indicate the moments of temperature change, which was sudden (Bělehrádek 1928*c*).

be expected that when a living system is cooled down to a given temperature, the velocity of its activities will not remain constant as the temperature equilibrium is established, but that it will continue to decrease for some time. Bělehrádek (1928*a*, *c*) has shown this to be the case in two instances studied by him; *viz.* in the heart-beat frequency of *Daphnia* (1928*a*) and in that of the larval trout (1928*c*). Fig. 30 is reproduced from the last mentioned paper. The temperature at which the time-factor appeared, was $+4.5^0$ in the first case and $+0.8^0$ in the second, and the time-factor observed was one to several hours and 20—40 minutes, respectively. It is interesting to note that on replacing the animals in the higher temperatures, the heart frequency returned to the normal without any appreciable time-factor. This feature also seems to exclude the possibility that the phenomenon might be ascribed to a slow thermal equilibration, or that it is due to the effect of accumulation of by-products in protoplasm.

It appears also that some data from earlier literature may be quoted in support of the view. Askenasy (1890) saw that the velocity of growth in germinating maize decreased 2—5 hours

after cooling the plants to a definite temperature. According to Ishikawa (1913), an *Amoeba*, when cooled down to 0° C, assumes a spherical shape only after one hour. Blanc (1916) has pointed out that the rate of CO_2-production in some plants depends not only on the temperature, but also on the time elapsed. A similar suggestion has been made by Kuijper (1910). Yet these findings in plants were criticised (de Boer 1927) as being due to imperfections of technique. They could also be ascribed to changes in chemical equilibria, which probably occur slowly. — Further similar instances will be quoted in IX. 5.

Yet it is desirable that a greater number of similar instances be presented before a definite conclusion is reached.

9. Temperature coefficients as indicators of protoplasmic viscosity

The other method suggested by Bělehrádek for testing the "physical" hypothesis is based upon the relation of viscosity to its own temperature coefficient. Heber Green (1908; compare Hatschek 1928, p. 112 seq.) has shown that the temperature coefficient of viscosity of saccharose solutions increases regularly with concentration and thus with viscosity at a given temperature (fig. 31). Although this rule may be con-

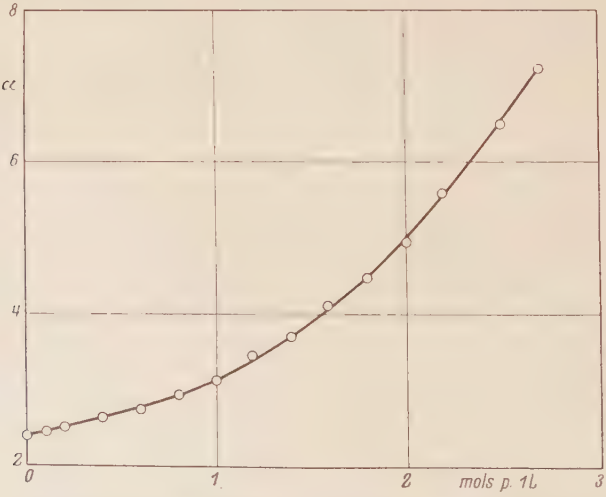


Fig. 31. Temperature coefficient (α) of the viscosity of sugar solutions increases with the concentration in sugar (abscissa) and thus with the viscosity itself (Heber Green).

firmed by numerous data available on various pure substances and solutions, no chemist has paid systematic attention to this phenomenon, neither is there any satisfactory explanation at hand[1]).

Bělehrádek (1929, 1932*b*) has given several examples computed from the work of J. Loeb on the viscosity of gelatin solutions as well as from the common tables of physical and chemical

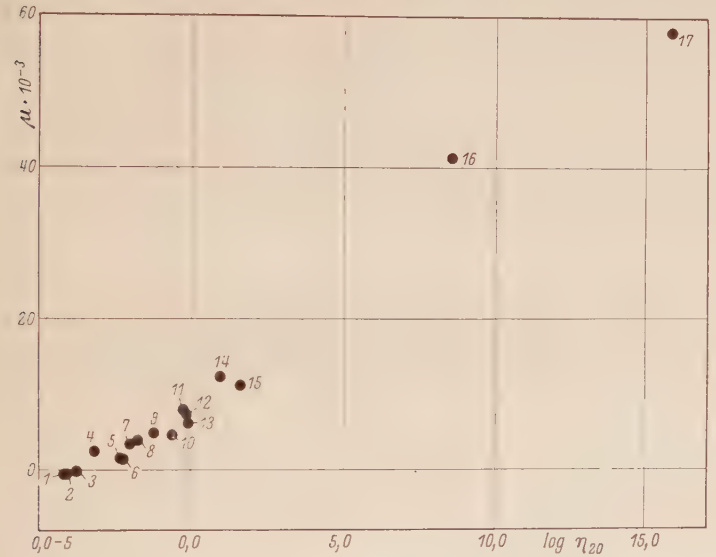


Fig. 32. Temperature coefficient (ordinate) of the viscosity of various substances as a function of the viscosity itself (on abscissa). 1: ether vapours, 2: gaseous hydrogen, 3: air, 4: liquid CO_2, 5: ethylacetate, 6: chloroform, 7: water, 8: 20% sacharose, 9: 40% sacharose, 10: sulphuric acid, 11: 60% sacharose, 12: 87% glycerine, 13: olive oil, 14: ricinus oil, 15: 90% glycerine, 16: pitch, 17: colophony (Bělehrádek 1932*b*).

constants of various substances. There are however many irregularities on the curve (fig. 32), but for a homologous series of substances or for a series of solutions of a given substance, the viscosity (at a given temperature) might be predicted, when its temperature coefficient alone is known.

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1) The writer is indebted to Mr. E. Hatschek, Cambridge, England, for this information.

It is important to note that an analogous relation has been established directly for the rate of diffusion and its temperature coefficient. H. S. Taylor (1924, p. 939—940) quotes from Kohlrausch (1908) that the temperature coefficient of the velocity of diffusion is apparently some function of the diffusion velocity itself, and that the greater the ionic mobility, the less is the temperature coefficient, and *vice versa*. Öholm has given the following figures to illustrate the relation between the rate of diffusion and its Q_{10}:

Table XXXII

Substance	Q_{10} of diffusion velocity
strong electrolytes	1.27
sugar	1.37
dextrine	1.41

Fig. 33 represents graphically another set of Öholm's data. According to Guldberg and Waage (quoting from H. S. Taylor,

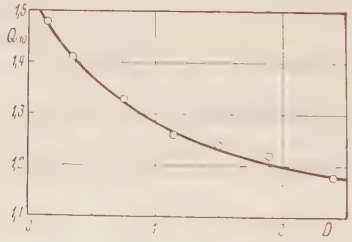

Fig. 33.
Q_{10} of the rate of diffusion in function with the rate (D) itself, according to Öholm.

1931), the temperature coefficient of the rate of dissolution of a metal in hydrochloric acid varies with the concentration of the latter thus:

Table XXXIII

Concentration (normality) of HCl	1.3	2.0	2.6	4.0	8.0
Rate of dissolution at 18°	1.58	1.68	1.70	2.44	3.25
Rate of dissolution at 0°					

This principle undoubtedly may be applied to the viscosity of protoplasm. It has been demonstrated in fact by F. and G. Weber (1916), that the temperature coefficient of viscosity

is high in cells whose cytoplasm is very viscous, while in those with low viscosity the coefficient is small. Therefore, if it is true that the temperature coefficients of vital processes are identical with or closely related to temperature coefficients of the diffusion velocity, which again depends on the viscosity, it must be found that all factors, which are known to modify the viscosity of protoplasm, will also modify the magnitude of temperature coefficients of vital reactions.

In fact we have seen in the preceding chapter that the magnitude of temperature coefficients in biology is often modified by various internal and external factors. As the protoplasmic viscosity has been found to depend on many such circumstances and actions, summarised by F. Weber (1922) and by Heilbrunn (1927), such a comparison can be made without difficulty. The necessary condition however is that a temperature coefficient be chosen which is constant for all experimental temperatures — a point which has been dealt with in detail in Chapter II.

Simultaneous modifications of the protoplasmic viscosity and of the temperature coefficients of vital activities take place under the action of the following factors (Bělehrádek 1932*b*):

(*a*) *Water content.* Investigations of B. Němec (1901), Heilbronn (1922), F. and G. Weber (1916) and Heilbrunn (1928, p. 212) have shown that both loss and excess of water increase the viscosity of protoplasm. In agreement with this, as we have seen (III.2), the temperature coefficient of the rate of development often varies with the water content of the organisms.

(*b*) *Electrolytes* exert considerable influence upon protoplasmic viscosity. According to Heilbrunn (1923) it seems that K increases the viscosity of protoplasm in Stentor, and an analogous increase in b can be stated for the heart activity (see III.3). It is probable that also other instances of the action of electrolytes upon the magnitude of temperature coefficient reported above (III.3) may be reduced to the changes of protoplasmic viscosity.

(*c*) *Narcotics* modify protoplasmic viscosity, as is now generally admitted, and they also change the numerical value of temperature coefficients, at least in some cases (III.4). This may also be applied to the action of CO_2.

(*d*) *Adrenaline* increases protoplasmic viscosity (Bauer 1926) and the Q_{10} of heart-beat frequency (Gellhorn 1926).

(*e*) *Caffeine* was found by Runnström (1928*b*) and by Bank (1932) to modify the viscosity of protoplasm, and by Bělehrádek (1932*a*) to affect the magnitude of *b* of the cardiac rhythm.

(*f*) *Light* seems to affect the viscosity of protoplasm to an important degree, as evidenced by the work of Weber (1929*a*). Accordingly there are now some instances known in which light modifies the temperature coefficient of vital processes (III. 8).

(*g*) *Age* is generally believed to induce an increase in protoplasmic viscosity. It has been demonstrated above (III. 11) that it also often modifies the temperature coefficients of reactions. In a few cases however the latter decrease with advancing age. This feature cannot at present be sufficiently explained, unless it is supposed that at least in some protoplasmic phases there is a decrease of consistency with increasing age. Albach (1928) has actually found a more rapid diffusion of some dyes in old plant cells than in young ones.

(*h*) *Mitosis.* The fact, now well established, that different phases of the mitotic process are associated with various protoplasmic viscosity (Chambers 1917, Heilbrunn 1921, Spek 1924, Kostoff 1930) is in agreement with the variations of the temperature coefficient in the course of mitosis (see III. 14).

(*i*) *Thermal adjustment.* It has been demonstrated above (III. 15) that the magnitude of temperature coefficients of analogous processes increases with the temperature to which the organism is adapted. Nobody as yet has demonstrated that a rise in protoplasmic viscosity would be associated with the process of thermal adaptation, though Hörstadius (1925) has suggested that a change in the dispersion of protoplasmic colloids takes place. Data will be analysed further on (V. 3) showing that protoplasmic fats and phosphatids are of a greater consistency when produced at higher temperatures. Thus when a given type of protoplasm is formed at different temperatures, its fatty substances will assume different physical properties and, because fats and phosphatids enter into the intimate composition of living matter, it is probable that the consistency of protoplasm, considered at a given temperature, will vary with the temperature at which it was formed. Details on this point are to be found in the original papers (Bělehrádek 1926*a*, *b*, 1931*a*).

(*k*) A fair agreement between what is known about protoplasmic viscosity and what may be expected from the "physical" point of view may be found also in the *injury* of protoplasm *by heating*. Russo (1910), Heilbronn (1914), Heilbrunn (1924*a*, *b*), Tirelli (1930*a*, 1931) Pigorini (1931) have shown that protoplasm or its ground-substance become more viscous on heating beyond the optimum, and from the data of E. and H. Gellhorn, quoted above (III. 16), it is evident that the Q_{10} of the permeability in frog skin rises considerably after a short exposure to 45°. On the other hand, when the normal cellular structure is destroyed by mechanical pressure, the temperature coefficient sinks considerably (see III. 16, Battelli and Stern).

It may be also that many individual, sexual and regional differences in temperature coefficients (III. 12, 13) will ultimately prove to be based upon analogous differences in protoplasmic consistency, as supposed by Bělehrádek.

In considering that the actual methods of direct viscosimetry can only evaluate the viscosity of the cytoplasmic ground substance and that there is no means at present to make even a rough estimate of the consistency of other protoplasmic phases, in considering further that various external and internal factors may not always modify the viscosity of these phases to the same degree or in the same direction as they modify the viscosity of the cytoplasm, there is some degree of agreement between the theory and the data. The chief difficulty this problem has to face however is the impossibility of comparing the protoplasmic viscosity with temperature coefficients of vital activities in one and the same type of protoplasm.

This point of view has been criticised by Crozier and Stier (1927*a*) and by Navez (1929, 1931), adherents of the "chemical" theory, to whom the notion of protoplasmic viscosity appears too vague, and by Stiles (1930*b*) who doubts whether the relation between viscosity and velocity of diffusion is as supposed by the law of Einstein and Smoluchowski. Stiles's objection has been dealt with above (p. 61). A more detailed answer to these criticisms together with further topics on the question must be read in the original (Bělehrádek 1932*b*).

There is however still another difficulty which has not yet been pointed out, *i. e.*, that in some instances the temperature coefficient rises with the velocity, whilst in theory the relation

should be just the opposite. Some of these cases have been quoted in connection with SKRABAL's views (IV.6). In such instances either different biochemical mechanisms must be supposed, or it may be that velocity is governed not only by the viscosity, but also by the concentration of the reactants. In fact the temperature coefficient of the rate of diffusion of various substances in water is independent of the concentration over a wide range from 0.01 to 1.0 or to 3.6 mols, as may be calculated from ÖHOLM's (1904, 1910) figures. As to the kinetics of the protoplasmic processes, the velocity may thus be increased chiefly by two mechanisms, namely (*a*) by increased velocity of single reacting molecules owing to a decrease of viscosity, or (*b*) by increased concentration of reacting molecules without simultaneous changes of viscosity. In the first case, the temperature coefficient will decrease, in the second case it will remain unchanged. By a combination of these factors the rate of the process may increase in spite of an increased viscosity, in which case the temperature coefficient too will increase.

The idea that the rate of biological phenomena is governed by the velocity of diffusion, cannot provisionally be accepted as more than a mere working hypothesis (BĚLEHRÁDEK 1929). Other ideas as to the temperature problem in biology must be viewed in the same light and it is still an open question which of them will prove more fruitful and more correct (J. GRAY, 1931; NEEDHAM 1931, p. 531). According to MAASS (1933), more facts speak to-day for the physical hypothesis than for the chemical one.

CHAPTER V

CHEMICAL PROPERTIES OF LIVING SYSTEMS AT BIOKINETIC TEMPERATURES

1. Action of temperature on chemical equilibria

In chemical processes and in adsorption reactions temperature acts in a twofold manner, *i. e.* it modifies the velocity of the reaction in a way which has been discussed in the preceding chapters, and secondly it affects the final or equilibrium state of the process. An excellent illustration of this double action is furnished by an experiment of W. M. Bayliss (1906), which it will be useful to reproduce here. When a piece of filter paper is immersed into a solution of Congo-red, it adsorbs the dye with a velocity which is dependent on temperature, and yields a $Q_{10} = 1.36$. But at the same time temperature modifies the maximum (final) amount of dye taken up. At low temperatures more dye is finally absorbed than at high ones (see fig. 34).

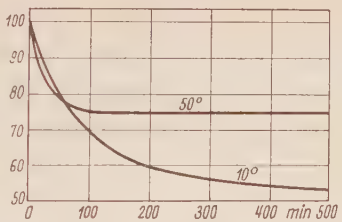

Fig. 34. Effect of temperature upon the rate of adsorption of Congo-red by paper. Abscissa: time in minutes, ordinate: total amount unadsorbed. Upper curve: at 50°, lower curve: at 10° (Bayliss 1906).

Speaking of chemical equilibria, one must distinguish between two cases:

(*A*) When the process by which a given substance is formed is accompanied by evolution of heat, *i. e.* when it is exothermic, the amount of substance ultimately present will be decreased by warming. Such is the case of Congo-red combined with paper.

(*B*) When the substance is with under absorption of heat, the process being endothermic, its final amount will be increased on heating. — In both cases the rate at which the reaction occurs is increased on heating.

As the position of equilibria depends on temperature and on some other factors, they are generally called "moving" equilibria. We owe our knowledge of moving equilibria especially to VAN'T HOFF, who also proposed an equation now usually written in the following form:

$$\frac{k_2}{k_1} = e^{\frac{q}{2} \cdot \left(\frac{1}{T_1} \cdot \frac{1}{T_2}\right)} \qquad \text{(XXX)}$$

where k_1 and k_2 are the final concentrations of the substance, reached at two (absolute) temperatures T_1 and T_2 respectively. The constant q is the number of calories developed or consumed during the reaction per one gram-equivalent of the substance formed. It has a negative sign in exothermic and a positive sign in endothermic processes.

It will be noted that the equation (XXX) has the same general form as the formula of ARRHENIUS for the velocity of chemical reactions (XV, p. 16). This similarity may easily be understood when we consider that in any chemical process two opposite reactions (combination and destruction) take place simultaneously, and that the amount of substances which is found at equilibrium depends on the relative velocities of both reactions. The equilibrium constant k at a given temperature is thus characterised by the relation between both reaction velocities at that temperature.

Although it is known that the amount of heat, which characterises chemical reactions, varies somewhat with temperature, it is evident that in reactions, in which the final state obeys this law, the numerical value of q will be found nearly the same for close temperature intervals. Yet it has been pointed out by PISSARJEWSKI (1908) that the viscosity of the medium modifies the position of chemical equilibria. Likewise many other factors, *e. g.* the chemical nature of the medium, radiations, pressure, etc. are known to alter the equilibrium in chemical processes (see, for instance, NATHANSOHN 1910). This fact seems to be very important in regard to any attempt to apply the law of moving equilibria to biological systems. It cannot be expected that it would be

possible to calculate q in biological processess, unless in the simplest ones, with a sufficient degree of reliability.

The most accurately studied case of moving equilibrium in biochemistry is that of oxyhaemoglobin, which is dissociated into haemoglobin and O_2 according to laws investigated by Barcroft and Hill (1910), Barcroft (1914), Mačela and Seliškar (1925). When haemoglobin is allowed to take up oxygen, it is partly transformed into oxyhaemoglobin and the final proportion of haemoglobin and oxyhaemoglobin varies with temperature, more oxyhaemoglobin being formed at low than at high temperatures. The equilibrium constant k varies with temperature as follows:

Table XXXIV

Temperature	Final concentration of haemoglobin	Final concentration of oxyhaemoglobin	k
16°	8%	92%	0.087
24°	29	71	0.408
32°	63	37	1.703
38°	82	18	4.775
49°	94	6	15.67

When $log\ k$ is plotted against the reciprocal of absolute temperature, a straight line may be drawn through the observed points, as required by the formula (fig. 35). Hence it can not

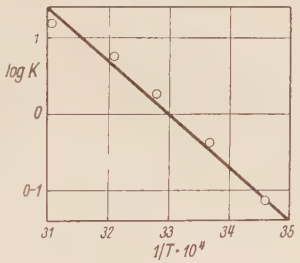

Fig. 35. Effect of temperature (abscissa: inverse of the absolute temperature) on the dissociation constant of oxyhaemoglobin (Barcroft and Hill 1910).

only be stated that about 28,000 calories ($= q$) are evolved when one gram-mol of haemoglobin unites with one gram-mol of O_2, but it may also be computed that the molecular weight of haemoglobin is about 16,000 (Barcroft 1914). But from a more recent study of Wastl and Leiner (1931) it seems to follow that q even in this simple case falls with increasing temperature, and that differences of pH somewhat modify its magnitude.

Even when it is not possible to evaluate directly the action of temperature upon the biochemical equilibria in living cells, it is at least to be concluded from what we know about the evolution of heat in reactions occuring *in vitro*, that many biochemical processes are but little dependent on temperature as regards the position of equilibria. Thus the enzymatic processes of hydrolytic character are associated with an insignificant production of heat and it may be concluded that their equilibria within the cell will be likewise not sensibly modified by temperature. Conversely the equilibria in cellular oxidations, which yield considerable amounts of "combustion" heat, must be supposed to be greatly affected by temperature variations.

In the subsequent paragraphs many instances will be given showing that the chemical composition of living systems is often changed by temperature. No attempt however can be made to calculate q of the respective processes, neither is it possible to state whether the chemical changes in question could or could not be conceived as being due simply to the direct action of temperature on moving equilibria. Such a possibility seems to be excluded *a priori* in considering that in a cell, as long as it is alive, no true equilibrium normally can be reached.

In the present state of knowledge no other statement can be made in regard to the moving equilibria in biology than that they in some way play a part in the general action of temperature on the protoplasmic activity (Kanitz 1907*a*, 1915, Robertson 1909, Demoll and Strohl 1909, Pütter 1911, Höber 1926, Jacobs 1928, etc.).

Some effects of extreme temperatures have been ascribed to changes in biochemical equilibria. This point will be discussed in another connection (IX. 8, X. 16*d*, XI. 10).

2. Water content

It is commonly known that hydrophilic gels when allowed to swell liberate heat, as has been first demonstrated by Duvernoy (for the bibliography, see Freundlich 1931, p. 580 seq.). But when swollen gels on further intake of water are transformed into sols, heat is again absorbed. Hence it will not be expected that the maximum water content reached by hydrophilic colloids at various temperatures would permit an accurate calculation of q. Neither may it be expected that temperature would

affect the maximum water content of colloidal systems in a uniform direction.

As regards living systems, it has long been known that red blood cells are richer in water at low than at high temperatures (see JACOBS 1928). According to HAUBERRIESSER and SCHÖNFELD (1913), the maximum amount of water taken in by a mammalian ligament is similarly increased by a decrease in temperature. Analogous results were obtained by LICHTWITZ and RENNER (1914*a*) in mammalian muscle and renal cortex, while the renal medulla behaved under the same experimental conditions in just the opposite way.

Contrary to these results E. ADLER (1921) finds in the muscles of a frog, that their water content is greater at high than at low temperatures. It is not yet possible to see whether this discrepancy is due to some regulatory process which might occur in muscles left *in situ*, as was the case in ADLER's experiments.

B. ILJIN (1922) was led by theoretical considerations to propose a formula to represent the interrelation between the maximum water absorption (capacity, $= C_\infty$) of living tissues and the absolute temperature (T):

$$C_\infty = C_0 \cdot e^{-\delta \sqrt{T}} \qquad \text{(XXXI)}$$

This equation seems to satisfy the results of HAUBERRIESSER and SCHÖNFELD and other results obtained with plant tissues. However it is not yet certain whether it would hold good in the majority of cases.

According to FAURÉ-FREMIET (1924), unfertilized eggs of *Sabellaria alveolata* undergo important changes in volume when the temperature is varied. Heating from 0° to 34° decreases the volume from 15.6 to 14.1×10^{-8} c. c. This change however is not continuous, but is interrupted between 18° and 25°, where no change occurs. The same temperatures represent the optimum of development. A similar relation between volume and temperature was obtained by EPHRUSSI (1927) with unfertilized eggs of *Paracentrotus lividus*, in which the volume remains constant between 19° and 24°. There seems however to be some discrepancy between these results and those of ACHARD (1927*a*, *b*) gained with the same material. According to this author, the volume of unfertilized eggs increases on heating from 20° to 35° and falls again above 35°. — In slime-molds, there seems to be no increase

of hydrophilic power of the protoplasm on cooling, as has been shown by Heilbronn (1922) by evaluating the viscosity.

Bodine (1921, 1923*b*) thinks that the low percentage of water which he has found in hibernating Orthoptera is due to temperature changes. He has found (1923b) that hibernating animals, when transferred to room temperature, not only resume their normal activity, but also increase their water content. Holmquist (1928*a*) has found that hibernating ants contain less water than under normal summer conditions, and that hibernating animals, kept in an ice-box until summer, give lower values than the controls. Seasonal differences in the water-content of insects have been claimed by Payne (1926—1928) as a cause of their cold-hardiness (see VIII. 2*c*). In the rice weevil, *Sitophilus oryza*, W. Robinson (1926) observed that on cooling the water content of the body first gradually increased, but decreased again before death occurred. According to E. F. and P. E. Adolph (1925), the water content of the earthworm, collected at 2°, increases on subsequent warming to 18°, the increase being roughly proportional to the logarithm of time.

In plants, some investigators attempted to determine whether the maximum amount of water, taken in by seeds, is dependent on temperature, but the results are far from being uniform. Dimitriewicz (1875) and Reinke (1879) stated that the maximum swelling of peas was independent of temperature between 0° and 50°, whereas the more recent experiments of Finikov (1926) show that in certain seeds the maximum imbibition increases with temperature.

Pantanelli (1920) has demonstrated that plant tissues, when submitted to temperatures a few degrees below zero, discharge water, and that this phenomenon is reversible as long as the tissue remains undisturbed by frost. Rosa (1921) and R. Newton (1923, 1924) have found that water is retained in wheat leaves collected in autumn and in winter with greater force than in those collected early in the fall. They think that during the cold season plant tissues are made more hydrophilic, and thus more resistant to freezing, by accumulation of colloidal substances with a high hydrophilic power. Similar ideas have been expressed also by other authors, whose work will be discussed below (VIII. 2*c*).

O. Hartmann (1919*c*) thinks that the water content of plant tissues is generally higher at low and lower at high temperatures,

and that it is determined by the lipocytic coefficient of the tissue, which also depends on temperature.

The most important studies in water content of plants however are those by A. Mayer and Plantefol (1925) undertaken on mosses (*Hypnum triquetrum*). At a constant tension of water vapour outside, the corresponding vapour tension within the tissue decreases at higher temperatures. In accordance with this phenomenon, water intake in mosses is accompanied by liberation of heat (A. Mayer, Plantefol and Wurmser). Prát and Minassian (1928) weighed different species of mosses left in dry air at two different temperatures (8^0—9^0, 18^0—20^0), but they did not detect any differences in the final weight.

It seems probable that the effect of temperature on the water content and on the hydrophilic power of living systems in general varies with the time of their exposure to a given temperature. After long exposures adaptive and regulatory processes may in some cases take place, which may cover the primary variations. This however applies primarily to the higher organisms.

Thus, however simple a property the water content of living systems appears to be, its dependence on temperature is not uniform.

3. Constitution of fats and phosphatids

It has long been known that mammalian visceral fat is more solid at a given temperature than subcutaneous fat. Henriques and Hansen (1901) have explained this by assuming that the melting point of fats varies with the temperature at which they were formed.

The melting point of fats depends, roughly speaking, on the degree of saturation of their fatty acids. Fats which contain bodies with many ethylenic (—CH = CH—) bonds, are more fluid and possess a lower melting point than fats with few or without such groups. Because the unsaturated groups react with iodine, the degree of non-saturation of a fat is easily detected by the "iodine absorption number". It may be said that the iodine number of natural fats and their melting point (or fluidity at a given temperature) show this supposed interrelation with a fair degree of accuracy (see for instance: André 1925, Kremann 1928, p. 236).

Many examples are now known which confirm the rule of Henriques and Hansen. These authors themselves have found the iodine number of natural fats from mammals and birds to decrease with increasing distance from the body surface and thus with increasing temperature at which they are formed. The cutaneous fat of an adult dolphin has a lower melting point than that of a foetus of the same mammal. The iodine number and melting point of mammalian natural fats also depend on the external temperature at which the animals have developed, as stated by Henriques and Hansen (1901) and by Lambling (1921). André (1925) has noted that the natural fats of marine animals, living at relatively low temperatures, are very fluid and relatively

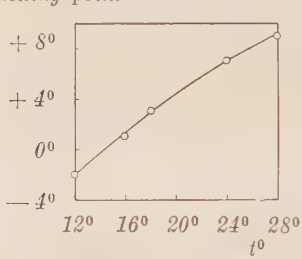

Fig. 36. Melting point (ordinate) of fatty globules in an Aphid, *Ropalosiphum prunifoliae*, in function with the temperature (abscissa) of cultivation (Ackerman).

rich in ethylenic and other unsaturated groups, as compared with those of land animals. Ackermann (1926) has cultivated aphids (*Ropalosiphum prunifoliae*) at different temperatures and has found that the solidification point of fatty globules in their bodies increases with the temperature of cultivation (see fig. 36).

The same rule applies to the vegetable fats, as pointed out by Czapek (1913), who compared the relative fluidity of vegetable fats from moderate climate with the relative solidity of tropical vegetable fats. Terroine, Bonnet, Kopp and Véchot (1927) confirmed the rule of Henriques and Hansen as regards natural fats of some animals and their eggs, and of certain lower plants. According to S. Iwanow (1929), the iodine number of linseed oil varies with the average temperature of the geographical region or with that of the hothouse. A recent publication of McNair (1929) contains an abundant list of various vegetable oils and fats with their melting points. Here, too, the general rule is roughly confirmed.

The cause of this particular behavior of natural fats lies, as pointed out by Leathes and Raper (1925) and by Pearson and

Raper (1927), in the general chemical laws which govern their formation. Fats in living systems are produced by a catenary series of chemical processes in such a way that unsaturated substances are formed first, and saturated ones are formed from these by subsequent transformations. At low temperatures this series cannot reach the stage of saturated fats, but is arrested at the stage of unsaturated combinations. At high temperatures however this same series proceeds further until saturated fats are formed.

The work of the authors just cited, which has been recently summarised in greater detail by Bělehrádek (1931*a*), concerns the animal and vegetable reserve fats. But it appears desirable also to examine from this viewpoint those fatty substances which form the protoplasm itself. It is known that fatty substances which are the integral part of living matter, differ from the reserve fats primarily by their relatively low melting point (see André 1925). More recent researches of Terroine, Hatterer and Roehrig (1930) have actually demonstrated that fatty acids of protoplasmic mono-amino-phosphatids give iodine numbers which are in perfect agreement with the theory. Their results are as follows:

Table XXXV

Species	Iodine number
(*a*) Monoaminophosphatids of seeds:	
Arachis	93
Linum	110
Soya	129
(*b*) Monoaminophosphatids of eggs:	
Birds	103
Tortoise . . .	106
Sea-urchin . .	135
Frog	142
Pike	163
Herring	208
Scallop	232

Apart from the qualitative changes produced by temperature in fats and phosphatids, important quantitative variations have also been described by A. Mayer and Schaeffer (1914*a*, *b*). According to these investigators, the amount of lipoid phosphorus

in frog's liver varies with the temperature at which the animals are kept, thus:

Table XXXVI

Temperature	Exposure	Average % of lipoid phosphorus (fresh weight)
2	8 days	0.097
23	8 days	0.102
24	8 days	0.144
26	2 days	0.196

In homoiothermic animals however the analogous relation is far from being simple, as is evident from further results obtained by the same authors.

It may be mentioned that Rywosch (1911), Heilbrunn (1924*b*) and Bělehrádek (1931*a*) have attributed the differences in sensitivity of various cells and organisms towards heat to the differences in melting point of their lipoid substances. The heat resistance of protoplasm thus seems to be "regulated" by the general property of fats and phosphatids to "adapt" their melting point to the temperature (see further X.17). From the same property, some conclusions have been drawn regarding the meaning of temperature coefficients of vital processes (see IV, 9).

4. Enzyme content

Burge and Leichsenring (1921) have found that the catalase content rises with temperature in the tortoise, and Burge (1923*a*, *b*, *c*) showed the same to occur in *Paramaecium* and in *Spirogyra*.

On the other hand, Lantz (1927) could not detect any analogous change in the catalase content of corn seed germinating at various temperatures.

5. Pigments

Besides pigmentation changes which occur in insects as a result of exposure to extreme cold or heat, some modifications of the pigmentation in this group of animals are also known to occur when temperature varies within the limits of the biokinetic zone (see especially Bodine 1921, Toumanoff 1926, Beljaev 1928, Alpatov and Pearl 1929, Zimmermann 1931). In a crusta-

cean, *Gammarus chevreuxi*, FORD and HUXLEY (1927) have found an increased formation of the eye pigment at higher temperatures.

That temperature may determine the deposit of pigment in the hair of mammals, has been demonstrated by W. SCHULTZ (1922—1930) for the Himalayan rabbit and by N. A. and W. N. ILJIN (1929) for the Siamese cat. This reaction, now commonly known as the SCHULTZ's reaction, consists in blackening of those parts of the hair which have been exposed to a low temperature. The threshold of effective temperature for the Himalayan rabbit lies between 1° and 30° and is different for various parts of the body (N. A. ILJIN 1926):

Table XXXVII

	Threshold of blackening
Nose	29°—30°
Tail	29°
Ears	25°—27°
Hind paws	16°
Fore paws	14°
Skin below eyes	2°—7°
Upper eye-lid	3°
Other parts	1°—2°

In the Siamese cat the thresholds are, according to N. A. and W. N. ILJIN (1929), distinctly higher:

Table XXXVIII

	Threshold for blackening
Ears	50°—60°
Paws	43°—45°
Back	30°—32° etc.

N. A. ILJIN (1926) showed that the thresholds of temperature depend upon age and race in the Himalayan rabbit, while in the Siamese cats the effect seems to vary according to sex (N. A. and W. N. ILJIN 1929).

W. SCHULTZ (1928, 1929, 1930) reports that he succeeded in inducing melanin formation in the normally albinotic eye-epithelium of the Himalayan rabbit, grafted to a cooler region of the

body, *e. g.* the ear, and that also the excised pieces of the albinotic skin, kept in humid air or in an oxygen atmosphere, showed a deposit of pigment in the growing hair follicles, when exposed to a lower temperature (35.5°) for several hours. At 38° no blackening occurs. It is probable that a definite minimum concentration of some substances is necessary for the melanin formation and that this concentration varies with the temperature according to the rule of moving equilibria, the substance being formed by exothermic processes.

The formation of some vegetable coloured substances seems to depend on temperature as well. As pointed out by O. Hartmann (1919*b*), higher temperatures seem to decrease the relative amount of chlorophyll. W. Schwartz (1930) has observed complete disappearance of chlorophyll in certain susceptible spots in the leaves of a variety of *Selaginella martensii*. In many plants red pigment appears on exposure to a low temperature (see Sachs 1860*b*, Pfeffer 1904, Davy de Virville 1925, Kosaka 1932).

The colour of certain pigments of vegetable origin is known to vary with temperature in a reversible way. Fitting (1912) proved that the change in colour with temperature in the flowers of *Erodium gruinum* and *E. ciconium* depends on the property of their natural colouring matter, which in watery solution changes its tint when the temperature is varied. The colour of flowers changes more quickly on heating than on cooling, whereas the extracted pigment reacts with the same rapidity independently of the direction of the temperature change. The substance is a pH indicator, but the described colour change has nothing to do with variations of pH, possibly induced by temperature (Fitting). Similar colour changes, due to temperature variations in vegetable anthocyans, were already known to Molisch 1889 (see Fitting 1912, V. Portheim 1914). — Certain complex salts of iodine, too, are known to change in colour when temperature is varied.

6. Electrolyte equilibria

As has been found by Hoagland and Davis (1923), cells of *Nitella* give up chlorides at 5°. When transported to room temperature, they again take up the lost chloride ion. Petrie (1927) has investigated the maximum (terminal) amount of diverse ions absorbed by slices of carrot at various temperatures, and has stated that increasing temperature favors the absorption of

anions and lowers that of cations. Substances experimented with were ammonium and potassium chlorides, ammonium sulphate and calcium nitrate. But GARDNER (1929) stated that the electric conductivity of cell-sap in pear shoots, which were kept constantly at 2°, was nearly the same as in shoots stored at 16°.

Temperature may affect also the degree of acidity (pH) of the cell-sap, as found by HURD-KARRER (1929) in the case of wheat leaves.

7. Apparent equilibria in metabolic processes

It was first found in plants that the amount and chemical nature of their metabolic products often depend on temperature. A. MAYER (1875) found that the tissues of *Oxalis corniculata* contain 13% of oxalic acid (reduced to dry weight), when the plants were kept at 20°, and only 8% when grown at 30°. The same acid is an abundant metabolic product in *Aspergillus niger*, but it is found only at low temperatures. At higher temperatures it disappears by oxidation (WEHMER 1891). According to GERBER (quoting from EULER 1909) the relative amount of various nutrient substances utilized by *Aspergillus* is not the same for different temperatures. At 12° the organism uses up more glucose and less tartaric acid, at 20° it utilises both sugar and acid in approximately equal amounts, while at 37° more sugar and less acid is taken in. As regards other acids, *Aspergillus* uses up malic acid at any temperature, while citric acid cannot be utilised unless at 30° and higher. v. EULER (1909), quoting this example, thinks that the described interrelation between temperature and the ability of certain organic acids to be utilised by plants is the reason why some fruits containing chiefly malic acid ripen at relatively low temperatures, whereas those which are rich in tartaric or citric acid, need higher temperatures for the ripening process. — For the earlier work the treatise of PFEFFER (1904) may be consulted.

During autumn and winter the starch reserves in trees are transformed into sugar and fat, while in March this process is reversed and starch regenerates from sugar and fat (see v. EULER 1909, IRAKLINOW 1912). The evidence that these transformations are induced by temperature changes was brought forward by experiments in which pieces of living wood and bark, transported in winter from outside to room temperature, showed a de-

finite starch formation within two hours (Büsgen, see Iraklinow). As pointed out by v. Euler (1909), these changes cannot be simply conceived as being due to variations of equilibrium states with temperature, because hydrolytic processes are known to be associated with a small caloric effect. Similar changes in woody plants are discussed in a recent paper by V. Novikov (1928). Yet it has been known for a long time that starch is transformed into sugar on application of low temperatures in other plants as well. The most common example of this is the case of potato tubers, studied first by Müller-Thurgau (1882), but the phenomenon was known already to Mer (1876, quoted from Åkerman 1927, p. 24) and has been often studied since (Lidforss 1898, 1907; Apelt 1907; Schaffnit 1911). Detailed discussion of the whole point is to be found in Åkerman (1927) and in Maximov (1929). — These and several other chemical transformations which take place in plants under the action of low temperatures are considered by some authors as being connected with hardening against frost (see VIII. 7).

Following Tottingham (1923), the amount of polysaccharids and of protein in certain plants seems also to depend on temperature.

Gardner (1929) has described the following chemical changes in pear shoots stored during the winter at $+2^0$, as compared with those maintained at room temperature: increase of hexoses, saccharose and organic acids, decrease of starch-content and of the freezing point of the sap. Water soluble polysaccharids (except starch), arbutine and nitrogen compounds showed no changes at all.

It may be remarked in this connection that the optimum concentration of nutritive substances sometimes varies with temperature. Thus Fulmer and collaborators (1921, 1924), Sherwood and Fulmer (1926) found the following optimum concentrations of ammonium chloride for the growth of yeast:

Table XXXIX

Temperature	0^0	10^0	20^0	25^0	30^0	40^0	43^0
Optimum % of NH_4Cl	0.0118	0.0236	0.0295	0.0319	0.0354	0.0413	0.0431

These data are represented graphically in fig. 37. — Temperature seems to modify in a similar way the optimum concentration

of certain nutritive salts in the case of root growth (S. F. and H. M. Trelease 1926).

In animal tissues much fewer instances of changes in metabolism with temperature have been described. Wechselmann (1921) and E. Adler (1921) have found an increasing lactacidogen content in muscles of frogs kept at a higher temperature. Brunow's (1911) researches on the metabolism of *Astacus fluviatilis* have demonstrated that at low temperatures up to 14° substances capable of decolorizing potassium permanganate are produced and that their excretion practically ceases at the higher temperatures.

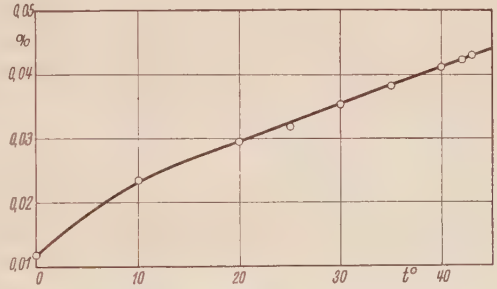

Fig. 37. Optimum concentration (ordinate) of ammonium chloride for yeast growth, as influenced by temperature (abscissa). (Sherwood and Fulmer 1926.)

More systematic studies of Scaffidi (1928) and collaborators (Gualdi 1928, Gualdi and Baldino 1930, Campo and Stanganelli 1928, d'Avanzo 1929) revealed important variations in the content of glycogen, lactic acid, fats etc. in cooled mammalian muscle and skin.

The respiratory quotient (CO_2/O_2) has been found by several authors to vary greatly with temperature. With increasing temperature it increases in value in yeast (Gréhout and Quinquaud 1888) and in the tortoise (Kayser and Ginglinger 1927), while in *Helix pomatia* (Pütter 1911) and in *Astacus fluviatilis* (Brunow 1911) it decreases. In embryos of *Aplysia* it is higher at lower temperatures, but only in very young stages (Buglia 1908). Some others have found that in certain plants the respiratory coefficient varies with (for literature see Tang 1932), or is independent of temperature (see Czapek 1913, III, p. 25).

Vieweger (1924) has observed in the leech an increased coefficient of assimilation of nitrogen, as expressed by the relation of protein taken in to nitrogeneous by-products, at higher temperatures. R. Hertwig (1903) observed that the protoplasm of Para-

maccium, cultivated at 8°, was stained in fixed preparations more deeply than in controls, which had been kept at 25°. This phenomenon seems to show that protoplasmic proteins undergo changes under the action of temperature, or that their combinations with ions are different at various temperatures. As a matter of fact, labile compounds of proteins with electrolytes are known to change their composition as the temperature varies (see KEITH LUCAS 1908).

However important may be the action of temperature in metabolic equilibria, it seems that temperature plays no part in the coefficient of utilization of energy in the living system, nor in the coefficient of utilization of nutritive substances for growth. This was demonstrated for the first time by RUBNER (1908) in *Proteus vulgaris*. HARTREE and HILL (1921) and COLLE (1929) stated the same to be true for muscle. TERROINE and his collaborators have multiplied the evidence for this general principle by several important contributions. TERROINE and WURMSER (1921) have shown that the amount of sugar used up to form one gram of dry substance in *Aspergillus niger* is the same for different temperatures. Germinating seeds of *Arachis hypogaea* and *Lens esculenta* show a similar interrelation (TERROINE, BONNET and JOËSSEL 1923). BARTHÉLEMY and BONNET (1924) have shown that the same rule applies to the growth of tadpoles. The relative amount of substance stored, in regard to that of substance burnt, here too is practically independent of temperature:

Table XL

Temperature	Time necessary to reach the stage when gills disappear	Coefficient of utilization of nitrogen
8°	30 days	0.75
10°	22 „	0.73
14°	20 „	0.75
21°	8 „	0.75

8. Apparent equilibria in other vital activities

It has been demonstrated by STRASBURGER (1878) that the zoospores of *Ulotrix*, which normally are positively phototropic, become negatively phototropic, when the temperature falls to a definite degree. Similar change in the sign of phototropic response has been observed by GROOM and J. LOEB (1890) in larvae of

Balanus, by J. Loeb (1893, 1908*b*) in those of *Polygordius* and in certain crustacea. These instances have been multiplied later on by the work of Dice (1914), Siedentop (1930) and others (see M. Rose 1929). The phenomena may be explained by the assumption that temperature modifies some chemical equilibria inside the cells, as first suggested by Demoll and Strohl (1909). J. Loeb (1893) has shown that the phototropic response changes in direction not only on cooling, but also on loss of water. Yet the phenomenon might also be explained as being due to some physical change in the protoplasm, *e. g.* adsorption, change in consistency, solubility, etc.

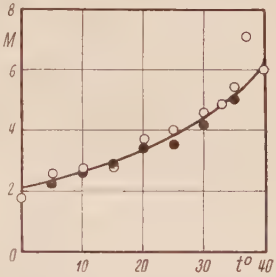

Fig. 38. Maximum degree of phagocytosis (ordinate) of bacteria by horse leucocytes, at varoius temperatures (abscissa). Madsen and Watabiki.

Madsen and Watabiki (1919), Madsen, Wulff and Watabiki (1919) have studied the action of temperature on phagocytosis in white blood cells. They have found that the maximum degree of phagocytosis, represented by the number of bacteria taken up per one leucocyte, decreases when the temperature is raised, as is shown by figure 38. It may be concluded that the process upon which phagocytosis is based is exothermic, *e. g.* an adsorption (see also Fenn 1922*a*).

In this connection it must be noted that the degree of contraction of various contractile systems is unlike for different temperatures. In many cases the degree of contraction increases on cooling, but this is not a uniform rule. For the details of this question, special treatises on physiology and the original papers must be consulted (degree or energy of contraction in striated muscle: Bernstein 1908, Harmon 1922, Wagner 1926, Fulton 1926*a*, Colle 1929; in heart muscle: Herlitzka 1905, Gellhorn 1924, Barlow and Sollmann 1926; contraction of melanophores in fishes: D. C. Smith 1931; length of pseudopodia in Rhizopods: Jensen 1902).

E. N. Harvey (1924) and Morrison (1924) have observed that the light emitted by bioluminescent organisms becomes more yellow as the temperature approaches or passes the limits of the biokinetic range.

9. Apparent equilibria in the action of substances on living systems

In the action of various chemicals upon living cells and organisms under varied temperature, two separate phenomena must be clearly distinguished, *viz.*, (*a*) the effect of temperature upon the rate at which a given substance permeates into the system or at which it produces definite changes in the system, and (*b*) the variations of the effective concentration, or of the final concentration, or of the mode of action, of such a substance, with temperature.

In this connection we shall deal with the second of these points. It has been now studied in a great number of instances and only the most important can be discussed here.

Much of the earlier work has been summarized by Zehl (1908), Kanitz (1915) and Roger (1933). In some substances the thre shold of concentration decreases with increasing temperature, whereas in others the relation is reversed. Especially substances which exert a typically narcotic effect belong to this second category (H. Meyer 1901, 1927; v. Knaffl-Lenz 1919; Collett 1923; see also J. Loeb 1906, p. 160).

Is has been known since Müller-Thurgau (quoting from Fuhrmann 1913) that the yeast cells stop their activity at a definite concentration of alcohol, which varies with the temperature thus:

Table XLI

t^0:	9^0	18^0	27^0	36^0
concentration (%) . .	9.5	8.8	7.5	3.8

This is in perfect agreement with what is now known of the action of alcohol and similar chemical bodies upon other organisms.

It may be found useful to tabulate the most important results gained hitherto by various workers (Table XLII).

It is interesting to note also that the action of hormones seems to depend on temperature in a similar way. According to Huxley (1929), a given dose of thyroid extract induces a complete metamorphosis in frog tadpoles (*R. temporaria*) only at a temperature of 5^0 or higher, whereas at a lower temperature the metamorphosis is incomplete, even when the tadpoles are afterwards transferred to a higher temperature. Also the effect

Table XLII

Substance	Toxic action: increases (+) decreases (—) does not change (0) on heating	Object	Authority
Acetone	+	moulds	Zehl 1908
„	+	tadpoles	H. Meyer 1901
Acids: boracic .	+	moulds	Zehl 1908
picric . .	+	„	„ 1908
Aconitine	+	frog	Veley and Waller 1910
Adrenaline . . .	+	heart of frog	Saéki 1925
„	+	„ „ „	Barlow and Sollmann 1926
Alcohols:			
ethyl- . . .	+	yeast	Fuhrmann 1913
„ . . .	+	tadpoles	H. Meyer 1901
„ . . .	+	moulds	Zehl 1908
amyl- . . .	+	„	„ 1908
isobutyl- . .	+	„	„ 1908
Atoxyl	+	frog	Sanno 1911
Benzylamide . .	—	tadpoles	H. Meyer 1901
„ . .	—	„	v. Knaffl-Lenz 1919
„ . .	—	various animals	Collett 1923
„ . .	—	moulds	Zehl 1908
Chloralhydrate .	+	tadpoles	H. Meyer 1901
„ .	+	various animals	Collett 1923
„ .	+	moulds	Zehl 1908
Chloroform . . .	—	„	„ 1908
Cocaine	0	frog	Saéki 1925
Colchicine . . .	+	„	Führer 1910, Sanno 1911
„ . .	+	bat	Hausmann 1906
Digitonine, small doses	—	blood cells	Bělehrádek 1932 *c*
Ether	—	moulds	Zehl 1908
Ethylurethane .	—	„	„ 1908
Guanidine . . .	+	heart of frog	Bělehrádek and Mirski 1932
Monoacetine . .	—	tadpoles	H. Meyer 1901
Sodiumbenzoate .	+	moulds	Zehl 1908

Substance	Toxic action: increases (+) decreases (—) does not change (0) on heating	Object	Authority
Sodiumoleate . .	0	blood cells	Bělehrádek 1932*c*
Phenol	+	moulds	Zehl 1908
Quinine	0	frog	Saéki 1925
„	+	moulds	Zehl 1908
Resorcine . . .	+	„	„ 1908
Salicylamide . .	—	tadpoles	H. Meyer 1901
„ . .	—	various animals	Collett 1923
„ . .	—	tadpoles	v. Knaffl-Lenz 1919
Saponin, large doses.	0	blood cells	Rywosch 1907
Sulphates of Al, Be, Co, Cu, Li, Ni, Zn	+	moulds	Zehl 1908
Sulphate of Ca .	+	pea	Cerighelli 1926*b*
Strychnine . . .	—	crab	Fröhlich and Kreidl 1921
Tetanus toxine .	+	frog	Courmont and Doyon 1893
„ .	+	bat	Koeninck 1899
Vanilline	+	moulds	Zehl 1908
Veratrine . . .	+	frog muscle	Lamm 1910
Veratrine, small doses.	—	„ „	Saéki 1925
Veratrine, larger doses.	+	„ „	„ 1925

of adrenaline depends on temperature (Sprinceana 1915; Barlow and Sollmann 1926).

In the case of haemolysis by saponin, Rywosch (1907) and Gorter, Grendel and Seeder (1931) could not state any relation between the temperature and the final degree of the process at a given saponin concentration, but Bělehrádek (1932*c*) has shown that when the concentration used is small, there is a net effect of temperature upon the final degree of haemolysis (see fig. 39). With increasing temperature the final effect decreases, thus suggesting that the underlying process is exothermic.

Similarly it has been demonstrated by Redfern (1922) that the final degree of *intra vitam* staining of carrot tissue is higher

at low than at high temperatures, the reaction being again of exothermic character, as may be expected in an adsorption process (see V.1).

The effect of some poisons is manifested by unlike physiological symptoms at various temperatures. Such is the case of alcohol, which in a definite dose produces muscular twitchings

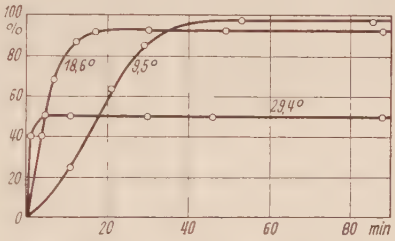

Fig. 39. Course of haemolysis of ox erythrocytes by digitonine 1 : 100.000 at three different temperatures. Abscissa: time. Ordinate: percentage of haemolyzed cells in the time (Bělehrádek 1932*c*).

in frogs at low temperatures, while at higher ones ($>18^0$) it manifests a narcotic action, as demonstrated by Pulcher (1925). It is probable that similar differences may be reduced to quantitative variations of toxic actions with temperature. — Compare further Roger (1933).

CHAPTER VI

VARIATIONS OF MORPHOLOGICAL EQUILIBRIA AT BIOKINETIC TEMPERATURES

1. Size of cells and of their constituents

R. Hertwig (1903) was the first to point out that the dimensions of cells and of their constituents, as well as the numerical value of the nucleo-plasmic ratio, are modified by temperature. His chief results on some protozoa were these:

Table XLIII

Temperature	*Dileptus anser*		*Paramaecium caudatum*,	*Actinosphaerium*,
	length (mm)	breadth (mm)	nuclear: plasmatic volume	diameter of a cyst (mm)
8°	0.510	0.112	1 : 7	0.090
25°	0.384	0.096	1 : 13	0.065

Similar results were obtained by Popoff (1908) on other protozoans, *Frontonia leucas* and *Stylonychia mytilus*. Here, too, the size of the body and nuclear volume increased on cooling and decreased on heating, while the nucleoplasmic relation showed a contrary change. The variations were completely reversible within one day. Rautmann (1909) observed in *Paramaecium* a decrease in the volume of the body with a rise in temperature and an increase in the nucleoplasmic ratio up to 20°. The action of temperature on the rate of division was different from that the on nucleoplasmic ratio. The time which elapsed between two divisions was sufficient to produce respective changes in animals transported into a new temperature.

RHUMBLER's (1911) studies on Foraminifera showed that only in species which do not form calcareous shells or in those which do so in a feeble measure, the body volume diminishes at higher temperatures, while in forms with a high calcium content it increases.

Variations of the cellular size in with temperature protozoans have been confirmed more recently by MICHELSON (1928) on *Paramaecium* and by E. F. ADOLPH (1929) on *Colpoda*. In both cases the body decreases in volume at higher temperatures, while

Fig. 40. *Ceratium*. a: "warm" form, b: "cold" form (KARSTEN).

in *Ceratium hirundinella* the relation is more complicated (HUBER and NIPKOW 1923: compare fig. 40, reproduced from KARSTEN 1928).

In *Stentor* the number of nuclei normally varies from one individual to another, yet the shape of the variation curve depends on temperature, as was demonstrated by v. PROWAZEK (1910). At 15° the peak of the curve is at 12 nuclear bodies, while at 25° it is only at 8.

E. F. ADOLPH (1929) has tried to explain the decrease of cellular volume with rising temperature in protozoans by the assumption that temperature stimulates the rate of division more than it does the assimilatory processes.

The first evidence that the cellular size varies with temperature also in metazoans, is due to VOINEA who, working under R. HERTWIG's direction (see R. HERTWIG 1903), found that the relative size of cells in newly hatched tadpoles of *Rana temporaria*, which developed at 10—11° and at 25°, was $1^1/_2$ and 1 respectively. CHAMBERS (1908) repeated these experiments on a larger scale, and found that the cells of different tissues in young frogs, cultivated from the egg at various temperatures, increased in size with a fall in temperature. O. HARTMANN (1918*a*) has confirmed these results in different amphibians, and has stated that higher temperatures induce also a diminution of the nuclear volume, of the number of nucleoli and of the nucleo-plasmic ratio. Yet different tissues reveal a different interrelation. The same author collected some other examples concerning the size of cells in

metazoans as dependent on the external temperature (O. Hartmann 1918*a*).

Diminution of cellular volume and of nuclear size at higher temperatures was demonstrated by several other authors in other groups of metazoans as well. It takes place in the sea-urchin (Marcus 1906, Erdmann 1908, Koehler 1912), in Cladocerans (Papanicolau 1910), in Entomostraca (O. Hartmann 1919*a*) and in eggs of Rotifers (Finesinger 1926).

The rule seems to hold good also in higher plants. O. Hartmann (1919*c*) gives the following figures for the pleromic cells in the pea:

Table XLIV

Temperature	Volume of the cell	Volume of the nucleus	Volume of the nucleolus	Nucleoplasmic ratio
8.5°	4312	354	61.8	0.0820
11.5°	4107	313	48.0	0.0752
18.0°	3000	209	35.7	0.0696
26.0°	3328	167	18.0	0.0501
31.0°	3796	102	19.3	0.268
37.0°	4896	344	20.5	0.702

It seems that each of these constants passes through a minimum value at a definte temperature, which is however different for the cell-volume and for that of the nucleus and nucleolus (fig. 41).

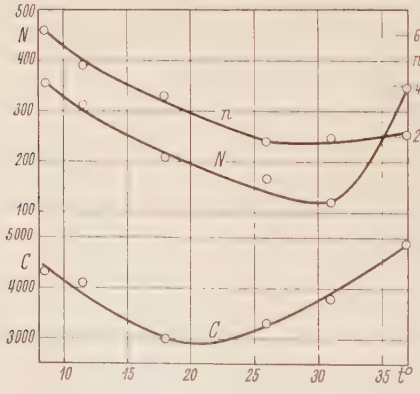

Fig. 41. Effect of temperature (= abscissa) on the volume of cell (*C*), nucleus (*N*) and nucleolus (*n*) in the pleromе of pea. O. Hartmann 1919*c*.

H. S. Fawcett (1921) states that at lower temperatures certain parasitic fungi (*Pythiacystis citrophthora*, *Pythophthora terrestria*) form thicker filaments than at higher ones, but that in other species just the opposite occurs. Maige (1923*a*) has measured the size of the nucleus and nucleolus in germinating beans with a result which

has confirmed the general rule. The diminution of nucleolar volume begins at 10° and continues progressively as the temperature is raised. When the plants are transferred to a new temperature, the nucleus assumes a new size, in accordance with that temperature.

Simultaneously with the changes of nuclear volume which take place under the action of temperature, ERDMANN (1908) stated also that the volume of chromosomes in the developing embryo of the sea-urchin changed noticeably. The volume of chromosomes decreases on heating, but the percentage of reduc-

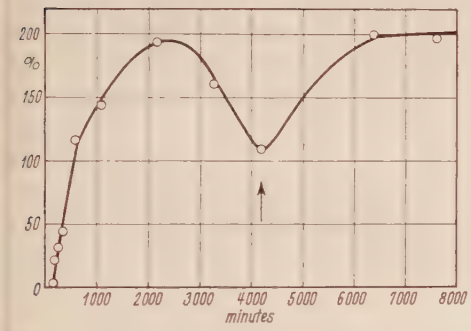

Fig. 42. Percentage increase (ordinate) of chromatin volume in sea-urchin embryos, on cultivation in cold, as a function of age (abscissa). The arrow marks the incipient gastrulation. (Computed from data of ERDMANN 1908.)

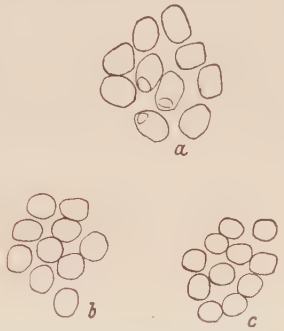

Fig. 43. Volume of chloroplasts in young leaves of *Helodea* cultivated at 0—2° (*a*), 20° (*b*) and 30° (*c*). O. HARTMANN 1919*b*.

tion for an elevation of temperature of 10° is not the same for different stages of development, as may be calculated from ERDMANN's figures. Temperature evidently has a more pronounced effect in the more advanced stages, but at the moment of gastrulation its influence temporarily falls. Figure 42 clearly shows this.

O. HARTMANN (1919*b*) grew *Helodea canadensis* at various temperatures and showed that the size of chloroplasts decreased rapidly at the higher temperatures (see fig. 43).

Here too the dimensions pass through a minimum at a certain temperature. According to HARTMANN, these changes may be conceived as being due to variation in cytological equilibria with temperature and are analogous to those detected in the nucleus and

nucleolus. — The size of cellular constituents seems thus to be connected by an interrelation which is under the influence of various factors, among which the temperature is probably the most important.

It is commonly known that the number and size of arms in Dinoflagellata depend on temperature (fig. 40) and that the seasonal variability in the morphological characters of these organisms may be reduced to this simple effect (see especially KARSTEN 1928; G. HUBER and NIPKOW 1923). Also the number and size of the food vacuoles in the ciliates depend on temperature (METALNIKOFF).

The explanation of why cells and nuclei should increase in size in protozoans, higher animals and plants at lower temperatures, is not easy. HARTMANN (1918*a*) admits several possible factors, among them chemical moving equilibria, changes in water content due to accumulation of osmotically active substances, accumulation of various substances whose solubility may be affected by temperature to an unequal degree, increased imbibition of colloids, variations of surface tension between the nucleus and the cytoplasm, changes in permeability of the nuclear membrane, etc.

2. Body size in higher organisms

Temperature affects the body size of higher animals more or less as in the protozoans and the cells of metazoa and metaphyta. This phenomenon, which however is not without exceptions, is important for the analysis of growth processes in general, as temperature may modify not only the velocity of growth, but also the maximum size of the body. For criteria of velocity of growth it is thus necessary to have recourse either to definite morphological changes, or, what is better, to the time needed by the growing organism to attain a definite part of the terminal size characteristic for the given temperature (see PRZIBRAM 1923).

The first to bring forward evidence that temperature acts upon the body size of higher organisms, was VERNON (1896). Sea-urchin eggs, which he cultivated at about 9° and at 25°, developed into plutei which were about 5 % shorter than those developed at 17°—20°. WO. OSTWALD (1904), who analyzed the general causes of seasonal variation in different groups of animals, took up the problem and proved in his experiments on Daphnids

that with rising temperature the dimenstions of organisms are diminished and thus that the seasonal variations of body size may be partly attributed to the temperature action.

Numerous investigators have since demonstrated the general validity of this rule: WOLTERECK (1909), PAPANICOLAU (1910), MCCLENDON (1910), AGAR (1913), RÜHE (1913), VOLTERRA (1926), MACARTHUR and BAILLIE (1929*a*) on Cladocerans[1]), ALPATOV and PEARL (1929), EIGENBRODT (1930) and IMAI (1933) on *Drosophila*, TITSCHACK (1925) on the moth (*Tineola bisellineata*), ABELOOS (1929) on *Planaria gonocephala*, JOHN (1933) on *Sagitta*, J. GRAY (1928*a*, *b*) on hatching embryos of the trout (*Salmo fario*), CHAMBERS (1908) and DOMS (1916) on frog tadpoles, etc. A rich bibliography on the subject is to be found in EIGENBRODT'S (1930) paper. It may suffice to quote here as an example the figures obtained by ABELOOS (1929) on the final body weight in *Planaria gonocephala*, reared at various temperatures:

Table XLV

Temperature	Final weight, mg
7°	53
12°	35
18°	25

Fig. 44 which is reproduced from GRAY'S (1928*b*) paper, demonstrates the twofold and opposite effect of temperature upon the growth velocity and upon the final body size.

ALPATOV (1929) quotes MICHAJLOV'S figures on the variation of body size in the honey bee in Russia with the geographical latitude, and shows that in the northern regions the body is smaller than in the southern ones, and that there is a continuous increase in body size

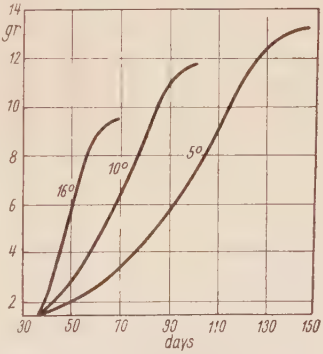

Fig. 44. Rate of growth and final size reached in trout larvae cultivated at 5, 10 and 16° respectively. GRAY 1928*b*.

1) MACARTHUR and BAILLIE found that only in males of *Daphnia magna* the final size of the body depends on temperature.

between the two extremes. He thinks that temperature, acting during the pupal stage, is one of the factors.

There are however some instances known in which temperature has no effect or even a contrary one upon the bodily dimensions. Thus European lizards are smaller in the north than in the south. Many tropical and subtropical forms attain greater sizes than the related forms in the moderate climate. Some examples were discussed by R. Hesse 1924.

As to the explanation, two different hypotheses have been put forward. Woltereck (1909) found in *Daphnia* that higher temperatures increased body dimensions when the nutrition was abundant. He is inclined to believe that a low temperature does not affect the body size primarily, but that it causes a relative deficiency of nutrition. McClendon (1910) holds the same view. Another explanation, offered by Pütter (1911, p. 181), is that the body size depends upon chemical equilibria which are modified by temperature.

3. Size and number of organs in higher organisms

If the ontogenetic development were caused by a single biochemical process, or by several such processes each depending directly on the other in their rates and final equilibrium stages, the terminal state might be altered by temperature only in a harmonious way, that is all the parts of the organism would change in the same ratio. Yet one may not expect that this should happen in reality, because the ontogeny depends upon many processes which do not always show a harmonious interdependence. In accordance with this, it has been demonstrated first by Vernon (1896) that the relative size of arms in plutei which had developed at various temperatures is not equal. Fig. 45 is computed on the basis of Vernon's results.

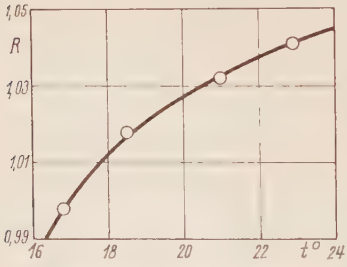

Fig. 45. Relative arm length (R) in sea-urchin larvae cultivated at various temperatures (t^0). Vernon 1896.

In fishes the number of vertebrae varies with the external temperature in such a way that cold favours a greater number (Hubbs 1922). The number of scales in the trout is subject to

analogous variations with temperature, as shown by MOTTLEY (1931). Seasonal variations in the relative size of the head in Cladocerans are probably caused by a similar temperature action (VOLTERRA 1926). A series of papers has recently been published dealing with the effect of temperature upon the number of eye-facets in *Drosophila* (ZELENY 1923, HERSCH 1924*a*, *b*, DRIVER 1926, 1931, LUCE 1931). It is interesting to note that the intensity of temperature action here varies from one race to another. According to HERSCH's figures the number of eye-facets is decreased, by a rise in temperature of 10° (between 15° and 25°), in the normal full-eyed race by 21.5%, in the "bar" stock by 59%, in the "ultrabar" one by 62%, and that the absolute number of eye-facets in these races decreases in the same order as the effect of temperature increases. This points to the possibility that the terminal number of eye-facets depends on the presence of some substances, the formation of which is subject to biochemical moving equilibria, easily displaced not only by temperature, but also by some hereditary factors.

NADLER (1926) and STANLEY (1931) found similar variations, caused by temperature changes, in the length of wing in *Drosophila*, but the response is different in each sex (HARNLY 1931).

It is commonly known that low and high temperatures favor the production of winged forms in some aphids (see ACKERMAN 1926). EIGENBRODT (1930) has observed that the number of circumocular hairs in *Drosophila* decreases with rising temperature. The paper of this author contains many other similar examples from older literature.

It seems that certain plants are also subject to similar variations (see *e. g.*, VERSLUYS 1928).

4. Sexuality and sex determination

There are several indications that temperature in some lower organisms decides between sexual and asexual or parthenogenetic reproduction. Thus KLEBS (1890, 1896) reported that thermal effects induce a formation of sexual cells in some algae, and also GREELEY (1902*b*) observed that *Monas* produced asexual spores at 1°—4°, while at 20° it divides both by the sexual and asexual method. The appearance of males among many rotifers, insects and crustaceans, too, seems to depend on temperature (WO. OSTWALD 1904, PAPANICOLAU 1910, SHULL 1930, TAUSON

1926, 1930, Whiting and Russell 1932), although other environmental factors may bring about a similar result (Shull). Also in moulds the method of reproduction seems to be determined by temperature (see Coupin 1930).

As regards the determination of sex, it has been demonstrated by Molliard (1898) that at higher temperatures female plants are produced in a larger proportion in *Mercurialis annua*. R. Hertwig (1906) has found that of frogs (*R. fusca*) produced at a temperature of 15°, about 75 % are males, while at 30° the number of males is 52 %. E. Witschi (1915), who repeated these experiments, found in a "differentiated" race of frogs a regular decrease of females with increasing temperature, as shown by the adjacent figure (fig. 46), while in an "undifferentiated" stock there were at 10° 438 females, 35 males and 18 intersexes, at 20° all females and no males. Witschi's results have been confirmed recently by J. Piquet (1930) on *Rana temporaria* and *Bufo vulgaris*. At 10° the final sex ratio is 1:1, at 20° the males are in majority, and at 25° there are only males and no females. Ovaries degenerate at higher temperatures and testes are formed instead. The most probable explanation is that given by Joyet-Lavergne (1930), who believes that higher temperatures establish a higher metabolic rate and variations in the nature of fats, thus inducing changes which are characteristic of the male sex. Temperature would thus act again in these cases in modifying biochemical equilibria connected with sex determination. — The somewhat different results of R. Hertwig are probably due to the fact that this author operated at 30°, a temperature which lies beyond the optimum for the frog (yet compare X.15, *f*).

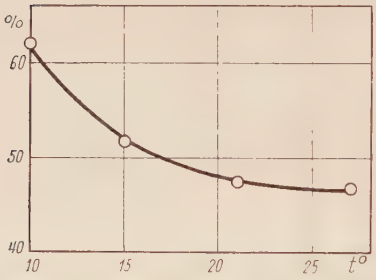

Fig. 46. Percentage of females (ordinate) in a stock of frog tadpoles kept at various temperatures (abscissa). Witchi 1915.

According to Seiler (1920), it is possible in a Lepidopteran, *Telaeporia tubulosa*, to obtain any desired sex ratio by varying the temperature. Here too the male sex is in excess at higher temperatures. The explanation given by Seiler is that during

ripening the sex chromosome tends to remain in the egg cell at higher temperatures and that it tends to be ejected into the polar body at lower temperatures. Presumably some physical change in the egg protoplasm may be responsible for this behaviour.

Important changes in sex ratio may be induced by temperatures which lie outside the biokinetic range. They will be discussed in Chapter X (15, *f*).

5. Size of populations

Since the size of cells and of organisms varies with temperature, it may be asked whether the same rule applies also to the final or equilibrium number of individuals of a given species living upon a confined substratum.

It is known in fact that plankton is much more abundant in arctic seas than in warm waters, and much discussion has arisen about the causes of this phenomenon (see especially J. Loeb 1908*a*, 1912, Demoll and Strohl 1909, Kanitz 1915, Oltmanns 1923). According to Lohmann (quoted from Oltmanns) the number of diatoms varies with the temperature of sea-water thus:

Table XLVI

Temperature	$<10^0$	15^0—20^0	25^0
Number of diatoms per litre of water	14,800	13,400	310

The graph 47, which is constructed from the figures of G. Smith (1920), shows an analogous relation between temperature and maximum density of bacteria. In Copepods however it seems that the maximum size of population is reached at the optimum of their development (Gause 1931).

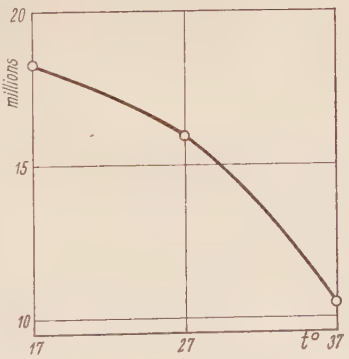

Fig. 47.
Maximum number of bacteria per standard loopful of cultivation fluid, attained at various temperatures. G. Smith.

CHAPTER VII

PHYSICAL PROPERTIES OF LIVING SYSTEMS AT BIOKINETIC TEMPERATURES

1. Protoplasmic viscosity

Much attention has been recently devoted to the action of temperature upon the viscosity of protoplasm. Yet it should be noted that the term is often understood from different points of view. Some workers adopt experimental methods in which viscosity is judged by morphological changes produced by centrifugation, plasmolysis, microdissection, etc., while others regard protoplasmic viscosity, as a factor which hinders free movements of the reacting molecules (IV.7—9). The difference between both points of view will perhaps become clearer in considering that a block of gelatin jelly may possess a high degree of mechanical viscosity and would not pass through a viscosimeter, yet many substances, *e. g.* electrolytes, diffuse through gelatin with about the same velocity as through water (Arrhenius 1892, Fürth and Bubanović 1918*a*, *b*, Stiles 1920).

To a chemist or a physicist the attempt of biologists to evaluate the viscosity of such a complex system as protoplasm or even one of its phases, must seem daring when compared with the ease with which the viscosity of chemically pure bodies or simple solutions can be determined. Even without this, when speaking about protoplasmic viscosity, much caution is necessary, as different parts of a cell do possess a different viscosity, which is very difficult to determine exactly by current experimental methods.

The first attempt to evaluate by experiment the action of temperature upon protoplasmic viscosity was made by B. Němec (1901). This author observed that starch grains in the root cells of *Vicia faba* moved under the action of gravity much more slowly at a low than at a higher temperature, and he explained

the phenomenon as a change in protoplasmic liquidity. In 1904, GREELEY stated that the consistency of the protoplasm in certain protozoans is increased by cold. A few years later, RUSSO (1910) observed an increase of the Brownian movement in the protoplasm of leucocytes and other cells of various species on heating up to a temperature optimum, beyond which a decrease again occurred. Similar observations were made by HOGUE (1910, p. 113) on the eggs of *Ascaris*. But the problem of protoplasmic viscosity in its relation to temperature did not attain a wider interest until the centrifuge method entered into biological practice. Yet we shall see below that many results gained by this method are subject to criticism.

FAURÉ-FREMIET (1913) found by the centrifuge method that the protoplasmic viscosity in the eggs of *Ascaris megalocephala* varies with temperature as shown by Fig. 48. In this special case, the effect of temperature on the viscosity is great and the temperature coefficient is high (Q_{10} between 8° and 18° = 5.0, between 18° and 30° = about 4.0).

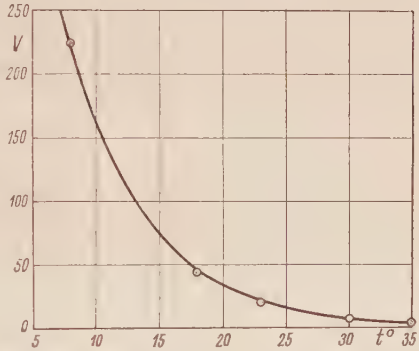

Fig. 48. Effect of temperature (t^0) upon protoplasmic viscosity (V) in the egg of *Ascaris*. FAURÉ-FREMIET 1925.

F. and G. WEBER (1916) evaluated the protoplasmic viscosity in the cells of *Phaseolus multiflorus* by the method of observing falling starch grains, and found a Q_{10} varying between 1.51 at lower temperatures and 1.14 at higher ones, similar to the results obtained for dilute solutions of proteins. Yet in cells whose protoplasmic viscosity was high, the temperature coefficient was equal to 2 or even more. This is in accordance with the general rule that bodies of high viscosity possess also a high temperature coefficient of viscosity and *vice versa* (IV. 9). Likewise WEBER and HOHENEGGER (1923) have observed a reversible increase in protoplasmic viscosity in plant cells on cooling, but their results do not make it possible to deduce the temperature coefficient.

Fauré-Fremiet (1924) observed that the protoplasmic viscosity in the eggs of *Sabellaria*, determined roughly by cytoplasmic movements on the application of pressure, varied between 0° and 40°. Pantin's (1924) results, obtained by the centrifuge method in the eggs of *Nereis*, similarly show a steady decrease of protoplasmic viscosity with increasing temperature (Table XLVII).

Table XLVII

Temperature	0.7°	10°	20°	30°
Relative viscosity	1.95	1.00	0.71	0.57

Likewise Derry (1929), working on cells of *Spirogyra*, has estimated that there is a minimum of viscosity at about 25° and that above and below this temperature a steady increase in protoplasmic viscosity occurs. Similar are the results of Thornton (1932) who worked on *Amoeba* with the centrifuge method.

From the work of all these authors it may be concluded that the protoplasmic viscosity shows a steady and continuous decrease when the temperature is raised up to the optimum. The temperature coefficient of this change varies in different kinds of protoplasm. Thus the action of temperature on the protoplasmic viscosity within the biokinetic range seems to be analogous to the effect of temperature on the viscosity of inanimate fluids (F. and G. Weber 1916). Similar steady progressive changes have been likewise observed by many experimenters in the viscosity of blood, blood serum, blood plasma (Snyder and Todd 1911, Lichtwitz and Renner 1914*b*, Lecomte du Nouy 1924) and of the liquid expressed from the eggs of the silk-worm (Pigorini 1924, 1931). The Table XLVIII gives the chief results as to the Q_{10} of protoplasmic viscosity.

Table XLVIII

Object	Authority	Q_{10}
Egg of *Nereis*	Pantin 1924	1.25 – 2.00
Hypocotyl cells of *Phaseolus multiflorus*	F. and G. Weber 1916	1.14 ± 1.51
Sap from eggs of silkworm .	Pigorini 1924	1.48 ± 0.03
Cells of *Spirogyra*	Derry 1929	2.04 ± 0.85
Egg of *Ascaris*	Fauré-Fremiet 1913	4.44 ± 0.56

On the other hand, HEILBRONN (1922) obtained quite a different kind of relationship in the plasmodia of slime-molds, in which he measured the protoplasmic viscosity with the aid of an electromagnet, in determining the force just sufficient to move a piece of iron introduced into the protoplasm. This author observed that the viscosity decreased both on heating and cooling the plasmodia, which were previously kept at room temperature. He concludes that it is not possible to determine the temperature coefficient of protoplasmic viscosity, which he supposes to be rather "regulated" by unknown cellular mechanisms.

Similarly HEILBRUNN (1920) observed that the cytoplasmic viscosity of fertilized sea-urchin eggs, tested by the centrifuge method, decreased on lowering the temperature to 10° or to the vicinity of 0°. Later on (1924*a*) the same author undertook more detailed investigations by the same method on the eggs of a marine mollusc, *Cumingia*. These studies revealed a rather complex type of relation between temperature and protoplasmic viscosity. According to HEILBRUNN, the curve representing this relation is W-shaped, showing two minima (at about 2° and 30°) and three maxima, one at very low temperatures in the vicinity of 0°, a second beyond 30° and a third at about 15°. In *Amoeba dubia*, another object studied by the same author (1929), the curve seems to be just as complicated (see fig. 49). Such a relationship between temperature and protoplasmic viscosity is difficult to understand and bring into line with the progressive change of viscosity with temperature, as observed by the other investigators. It is also difficult to conceive why such phenomena which evidently depend on protoplasmic viscosity, as the ameboid movement or protoplasmic streaming, show a progressive change in velocity with varying temperature (compare HEILBRONN 1922, PANTIN 1924, LAMBERS 1925). As to the explanation, RUNNSTRÖM (1928*b*) suggested that at 15—16° there is a maximum of mutual attraction of the colloidal particles, an assumption which however remains to be demonstrated. HEILBRUNN himself gives no explanation, but he claims that similar results have been observed by other investigators in various types of cells. There are first the data of HEILBRONN on the viscosity of slime-molds, which, as we have just seen, seem to possess a maximum viscosity at room temperature. In considering however that HEILBRONN operated only at one low

temperature (12°) without extending his investigations to several other temperatures of the biokinetic zone, it is highly questionable whether one is authorized to draw any such conclusion from his results. In addition, HEILBRONN stated that the protoplasmic viscosity in slime-molds is greatly modified by variations in water-content and that the latter increases with

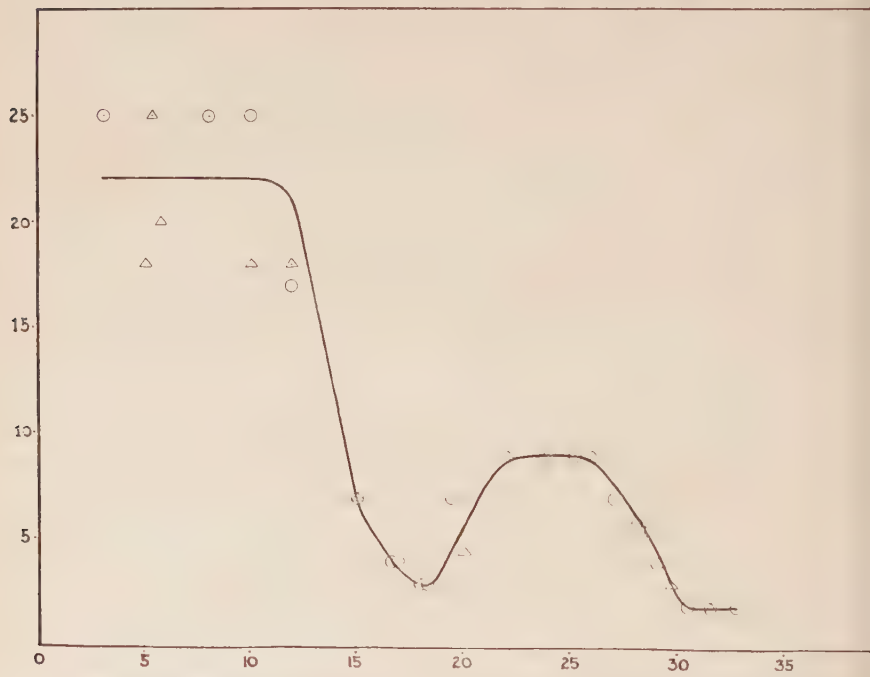

Fig. 49. Effect of temperature upon protoplasmic viscosity in *Amoeba dubia*. HEILBRUNN 1929.

sinking temperature. In his measurements one faces therefore two opposite effects of temperature, namely the direct action of temperature on the viscosity and an indirect temperature action on the water-content and thus on the viscosity.

There are further more recent investigations by BAAS-BECKING, VAN DEN SANDE-BAKHUYZEN and HOTELLING (1928) which, too, have been claimed by HEILBRUNN (1929) in support of his views. These authors attempted to evaluate more accurately the protoplasmic viscosity in the cells of *Spirogyra* by

observing the amplitude of the Brownian movement in the external layers of cytoplasm. Unfortunately the results of these investigators were rendered unreliable by an unexpected inconstancy of the speed of the Brownian movement at one and the same spot at different times. In one of the most "constant" spots, they found values ranging from 0,052 to 0,250 (expressed in arbitrary length units), but usually the differences were still larger. For this reason the standard error of the means is very high (table VI in the quoted paper, p. 26) and it cannot be said with certainty to what extent the results of the above mentioned authors depict the true viscosity of the cytoplasm.

As to the experiments of HEILBRUNN (1920—1924) on eggs, they have been criticized by several biologists, chiefly by SPEK (1928). First it is known that the eggs of some marine animals undergo a spontaneous stratification during the process of maturation (see *e. g.* SPEK 1931, BANK 1931, 1932) and some of them can be forced to such stratification by external agents. It is of special interest to note that PAYNE (1930) found a stratification analogous to that which occurs in centrifugation, in the eggs of *Cumingia* on simply reducing the temperature. These results are obviously of such a character as to make the use of the centrifuge method in evaluation of viscosity changes with temperature in ova of certain marine animals highly problematical. BANK (1931*b*, 1932) stated that the eggs of the sea-urchin, treated with caffeine, show a "spontaneous" stratification which is analogous to that induced by the centrifugation not only in its external appearance, but also in its dependence upon certain internal factors, so that the eggs which are easily stratified by centrifugation, likewise stratify spontaneously with the application of caffein. There is therefore every reason to supose that the centrifuge method cannot in similar cases yield reliable results unless all the secondary factors, which according to GRAY 1931 (p. 66) and BĚLEHRÁDEK (1932*b*) probably enter into play, are more accurately known. Special caution is necessary when conclusions are drawn from such results as to the temperature action on the protoplasmic viscosity. This applies in particular to the experiments of HEILBRUNN, in which the viscosity tests were made after, and not during, the exposure of the eggs to the temperature concerned, so that their real temperature certainly differed from that which was measured before their centrifugation.

HEILBRUNN himself (1928, p. 110) very clearly recognizes this source of error in his technique.[1])

HEILBRUNN (1929) performed another set of experiments on *Amoeba dubia*, in which he similarly estimated the cytoplasmic viscosity as being connected with the time necessary to shift crystalline granules of the body to one pole. The result again was a curve with two minima (one between 15° and 20° and the other at about 30°), a maximum in the vicinity of 25° and a plateau beginning at 5° and dipping at 15°. On inspection of the curve (fig. 49) it is obvious that the left side of the curve from 15° downwards is not very convincing, owing to the considerable dispersion of the observed points. The right side of the graph should be interpreted with great reserve. As pointed out by HEILBRUNN, the crystalline bodies in this type of *Amoeba* accumulate in the vicinity of one pole, even without centrifugation, at higher temperatures, and it is not at all possible to determine to what extent the results are affected by this secondary factor.

In a recent investigation, ROMIJN (1931) tried to determine the consistency of the protoplasm in *Nitella flexilis* from the shape which the protoplast assumes when plasmolysed, and he found a maximum of consistency at 21°—22°. When it is considered however that the method adopted by ROMIJN is not sufficiently accurate to allow of a quantitative determination, and that the simultaneous effect of protoplasmic adhesiveness (VII. 2) is not excluded, no definite conclusion may be drawn from his observations.

It is obvious that much more work is to be done before it will be possible to obtain a clearer idea about the viscosity of protoplasm and its dependence on temperature.

In hydrophilic colloidal solutions the viscosity at a given temperature depends also on the time elapsed from the moment when the solution attained the temperature equilibrium. This time-factor, which seems to be a characteristic feature of hydrophilic colloids, and which is practically absent in true solutions, is largely known chiefly from the experiments of J. LOEB (1922), on gelatin solutions. It is thus to be expected that the viscosity of protoplasm at a constant temperature too will depend on the

1) See also COSTELLO, 1934.

time-factor. However so far no such investigations have been undertaken (see WEBER and HOHENEGGER 1923 COSTELLO 1934).

2. Adhesiveness of protoplasm

The adhesiveness of protoplasm is usually determined as the degree of difficulty with which a cell, moving on some solid substance, releases itself from the latter, or, alternatively, the degree of ability of plant protoplasts to adhere to the cell walls when plasmolysed. It was known as early as 1885 to H. DE VRIES that the tendency of plant cells to assume a round shape, when plasmolysed, is increased at higher temperatures. The same phenomenon has been demonstrated more recently by WEBER and HOHENEGGER (1923) on *Spirogyra*. FENN (1922*b*) has found in white blood corpuscules that the percentage of cells which adhere to glass is greatest in the vicinity of 20°. In the degree of adhesiveness there is a considerable time-factor, whose length varies with temperature.

The adhesiveness of protoplasm is in some way associated with its viscosity (compare PFEIFFER 1933; CHOLODNY and SANKEWITSCH 1933), yet it would be premature to confuse the one with the other or to regard them as parallel phenomena. The chief reason for such caution lies in the fact that the viscosimetric methods take only the cytoplasm into account, whereas the adhesiveness applies only to the cellular surface, whose properties differ greatly from those of the interior of the cell. It is thus correct to speak of the adhesiveness and the viscosity of living matter as two separate qualities, as does *e. g.* FENN (1922*b*). FAURÉ-FREMIET (1929) has actually demonstrated that the adhesiveness of the amebocytes of a starfish is small at 0°—5°, while it must be expected that the protoplasmic viscosity will be high at such a temperature.

3. Elasticity and other mechanical properties

As it is known that the thermal coefficient of change in volume or length in colloids is related to the colloidal structure, it is interesting to investigate these constants in living tissues. JENSEN (1909) found that a normal resting muscle shortens progressively on warming and lengthens on cooling. WÖHLISCH's (1926) investigations on a living elastic ligament yielded more detailed results. This author measured (*a*) the cubical thermal

coefficient of dilatation, which indicates the volume change (reduced to the actual volume at 0°) taking place on heating 1°; (*b*) the linear thermal coefficient of dilatation, which indicates in an analogous way the change in length or in breadth. In anisotropic bodies, however, this coefficient is not the same for longitudinal and transverse dilatation. The coefficients found by Wöhlisch besides are not equal for different temperatures, and at very high temperatures even their sign varies:

TABLE XLIX

	Thermal coefficient of dilatation						
Temperature	10°	20°	30°	40°	50—60°	65°	70°
longitudal	—0.0044	—0.0038	—0.0021	—0.0010	0.0007	—0.0023	—0.0031
transversal	+0.0034	+0.0031	—0.0022	—0.0015	- 0.0008	—0.0171	—0.0067
cubical	+0.0024	—0.0024	—0.0023	—0.0020	+ 0.0009	—0.0365	—0.0165

A similar behavior is observable in rubber and in some crystallized inorganic substances (see Wöhlisch 1926 for further details and for previous literature).

From the experiments undertaken by Hieronymus (1913) on the frog's sartorius it may be concluded that the modulus of elasticity increases slightly at higher temperatures:

TABLE L

Load (*g*)	0	2	5	10	20	40
Length at 5.5° . .	100	104.4	108.7	113.0	115.2	117.4
Length at 12.5° . .	100	105.5	112.9	116.6	118.9	120.4

A similar result has been recently noticed by Colle (1929), de Nayer and Bouckaert (1931).

4. Osmotic resistance.

According to Hamburger (1886) red blood cells lose haemoglobin in hypotonic solutions at a definite osmotic concentration, which is independent of temperature. His experiments however were criticized by Jarisch (1921) who found on the contrary that haemolysis takes place within 15—20 minutes at a threshold concentration of NaCl, which is not equal for different temperatures, being higher at low and lower at high temperatures. There

is a maximum resistance (characterized by a minimum threshold concentration) at a definite temperature, which varies from one species to the other. The change of resistance according to temperature is reversible, in view of the fact that erythrocytes, which showed no haemolysis in 0.44 % NaCl at 44°, are readily haemolyzed on cooling. Addition of neutral phosphates increases not only the resistance but also the influence of temperature exerted on it. This influence varies in the examined cells of various mammals in the same sequence as their natural resistance and as their phosphate content. (See further JACOBS and PARPART 1931.)

5. Bioelectric potentials

In a reversible galvanic cell the electromotive force obeys the law of GIBBS and HELMHOLTZ, being proportional to the absolute temperature. BERNSTEIN (1902) examined the magnitude of the P. D. (potential difference) of the action currents of muscle and nerve at various temperatures and arrived at the conclusion that there is a fair amount of agreement with the membrane theory of bioelectric currents. As a matter of fact, it was known to several authors previous to BERNSTEIN that in some cases the P. D. of bioelectric phenomena increases with the temperature up to a definite optimum: ENGELMANN (1872). BACH and OEHLER (1880). V. GENDRE (1884; P. D. of frog skin). Likewise WALLER (1904) found that in a cat's foot, whose nerve was stimulated, the P. D. was 0.0065 volts at 9° and 0.0122 volts at 35°. Yet the proportionality between P. D. and absolute temperature was not established by LESSER (1907) for the frog skin. Recently LUND and MOORMAN (1931) found that the P. D. of frog skin varies with temperature to a much more important degree than postulated by BERNSTEIN's hypothesis, and a similar statement has been made by UMRATH (1934) for the action currents in *Nitella*.

Analogous phenomena in plants have been studied by HAAKE (1892), PLOWMAN (1903) and BOSE (1907). The electromotive force of the current of injury has been found to decrease on cooling and the current may even change in direction at low temperatures.

There is strong evidence that in many other bioelectric phenomena the P. D. increases on cooling and decreases on heating. BURDON SANDERSON (1899) observed this to be the case in the action currents of the frog sartorius muscle, and also GARTEN

(1901) described a similar effect with the same object, though the difference was but slight. From the results of K. Lucas (1909) on the action currents of the muscle it may likewise be seen that the P. D. of the diphasic wave increases on cooling:

TABLE LI

Average maximum P. D. in volts at	
8°	18°
+0.0478	+0.0405
—0.0669	—0.0476

Kolb's (1928) results obtained with a frog's nerve also show a decrease in P. D. with a rise in temperature. As far as the discharges of electric organs in fishes have been measured in relation with temperature, it seems that their electromotive force too increases on cooling and *vice versa* (Garten 1910*a*, *b*; Koike 1910).

The action of temperature upon the P. D. of bioelectric phenomena is thus far from being uniform. Bioelectric currents may be accordingly divided into three groups:

(*a*) The P. D. increases in proportion to the absolute temperature, the change in P. D. being small.

(*b*) The P. D. increases on heating much more than to merely remain proportional to the absolute temperature.

(*c*) The P. D. decreases on heating and increases on cooling.

The third of these groups seems to be the most numerous, as far as present knowledge goes. Even when it is taken into account that the electric conductivity of cells and tissues increases on heating (see VII. 6), and that temperature variations affect not only the actual P. D. but at same time the electric resistance, the discrepancies in the results are too great to enable one to reduce all the phenomena concerned to a uniform basis.

6. Electric conductivity

As regards its relation to temperature, the electric conductivity of living systems behaves in a way which is analogous to the conductivity changes in solutions of electrolytes. The following table may suffice to illustrate this:

TABLE LII

System	Q_{10}	Range of temperature	Authority
KCl in water	1.22—1.24	18—25°	computed from Noyes
NH_4Cl in water	1.23	18—25°	computed from Noyes
NaCl in water	1.25	10—20°	Osterhout 1914
Sea-water	1.26	10—20°	Osterhout 1914
Laminaria sacharina (dead)	1.26	10—20°	Osterhout 1914
Laminaria sacharina (liv.)	1.32—1.34	10—20°	Osterhout 1914
Leaves of ivy (*Hedera helix*)	1.31	10—20°	Dixon and Bennett-Clark 1928
Leaves of ivy (*Hedera helix*)	1.38	20—30°	Dixon and Bennett-Clark 1928
Leaves of ivy (*Hedera helix*)	1.13	30—40°	Dixon and Bennett-Clark 1928
Leaves of *Aloe* (dead) . .	1.21	20—30°	Sen 1928
Leaves of *Aloe* (dead) . .	1.18	30—40°	Sen 1928
Leaves of *Aloe* (living) . .	1.25	20—30°	Sen 1928
Leaves of *Aloe* (living) . .	1.12	30—40°	Sen 1928
Pulvinar of *Mimosa* (dead)	1.16	25—35°	Sen 1928
Pulvinar of *Mimosa* (dead)	1.07	35—45°	Sen 1928
Pulvinar of *Mimosa* (living)	1.29	25—35°	Sen 1928
Pulvinar of *Mimosa* (living)	1.05	35—45°	Sen 1928
Leaf of *Aloe* (dead) . . .	1.22	15—35°	Bělehrádek and authors 1935
Leaf of *Aloe* (living) . . .	1.48	15—35°	Bělehrádek and authors 1935
Liver of the frog	1.15—1.34	15—25°	Bělehrádek and authors 1935
Brain of the rat	1.29	10—20°	Bělehrádek and authors 1935
Brain of the rat	1.17	20—30°	Bělehrádek and authors 1935
Visceral fat of the cow .	1.32	15—25°	Bělehrádek and authors 1935
Visceral fat of the cow .	1.41	25—35°	Bělehrádek and authors 1935
Cartilage of the calf . . .	1.26	20—30°	Bělehrádek and authors 1935
Centrifuged blood cells (pig)	1.31	20—30°	Bělehrádek and authors 1935

Even in considering that the determination of electric conductivity of living systems is not a simple problem and that moreover the electric capacity of tissues usually hinders the measurement (GILDEMEISTER 1928), the results tabulated above may still be taken as fairly reliable. In fact there is very close agreement between living systems and simple salt solutions.

OSTERHOUT (1914) showed that the temperature coefficient of electric conductivity is somewhat smaller after the death of the plant tissue. Similar results were obtained by SEN (1928), and by BĚLEHRÁDEK and collaborators (1935 — see the above table). This may be explained by the fact that in dead tissues the exchange of electrolytes becomes more rapid owing to the post-mortem increase in cellular permeability, and that the temperature coefficient of the rate of diffusion varies with the rate itself, as has been already pointed out in IV. 9.

CHAPTER VIII

FREEZING AND FROST RESISTANCE

1. Freezing and supercooling

When a watery system is cooled to its freezing point, ice formation takes place. Under certain conditions, however, the system may be cooled even below that point without ice formation, which may occur subsequently on further cooling or on changing the conditions of the system. In such a case heat

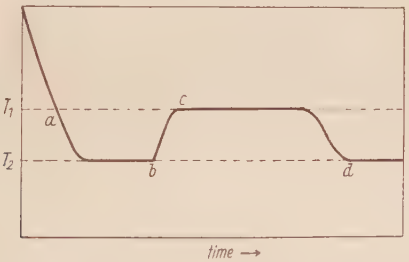

Fig. 50. Supercooling of a body, illustrated on an ideal case. Abscissa = time, ordinate = temperature. T_1 = freezing point of the body, T_2 = temperature of the bath. From the moment *a* the body, instead of freezing, passes into a supercooled state, which is disturbed in the moment *b*. The body begins to freeze and evolves heat, owing to which it maintains a temperature identical with its freezing point, from the moment *c* onwards. When the body has completely frozen, its temperature falls again untill it becomes equal to the temperature of the bath (*d*).

is produced and the system freezes, and assumes a temperature which is equal to its freezing point and which does not fall again until all the water is transformed into ice.

Phenomena of supercooling have often been described in living systems and also some of their causes are known. Figure 50 represents the course of an ideal case. More details are to be found in Kodis (1898), Bachmetjew (1899—1901), Bartetzko (1910), H. W. Fischer (1911), P. Jensen (1913), Höber (1924), Åkerman (1927), Tigerstedt (1910—14), W. Robinson (1929) etc.

Different authors have often observed a very unequal frost resistance in one and the same type of organism. This is obviously due to the fact that the degree of supercooling varies with many external and probably also internal conditions, which often remain undetermined (Höber 1924). Much caution is therefore necessary in estimating frost resistance in organisms, and in comparing data gained by various investigators often under unlike conditions. Even when ice crystals are found around the tissue or in the free spaces in it, and when the tissue assumes the external appearance of a frozen body, it is not yet proved that the tissue itself is frozen. Many investigators have long agreed that death occurs in cells inside of which ice formation has taken place, and that no ice was really formed inside the living matter when a recovery from a frozen condition was noted (see especially Pouchet 1866, Kodis 1899, Pictet 1893*a*, *b*, Bachmetjew 1899—1901, H. W. Fischer 1911, P. Jensen 1913, Cameron and Brownlee 1914, Höber 1924, Payne 1926*b*, 1928, Weigmann 1929).

The freezing point of protoplasm and of the organic liquids depends obviously on the presence of crystalloids dissolved, and varies therefore to a great extent. May it suffice to quote here the following data:

TABLE LIII

System	Freezing point	Authority
Rana fusca (whole)	—0.54°	Weigmann 1929
Lacerta agilis (whole).	—0.68°	Weigmann 1929
Rana pipiens (whole)	—0.44°	Cameron and Brownlee 1914
Blood of hen	—0.607°	Atkins 1909
Blood of goose	—0.552°	Atkins 1909
Egg juice of silkworm	—0.6°	Pigorini 1931
Same at the end of hibernation .	—2.0°	Pigorini 1931
Frog's muscle	—0.45°	P. Jensen 1913
Frog's blood	—0.44°	Urano 1908
Cell-sap, *Aloe arborescens* . . .	—0.14°	Bottazzi 1925
Cell-sap, *Agave americana*. . .	—0.52°	Bottazzi 1925
Cell-sap, *Sedum maximum* . .	—0.44°	Bottazzi 1925

In the majority of instances the freezing point lies only slightly below 0° C. Yet the tissues may be supercooled to a

considerable degree, *e. g.* a frog muscle to —10° or —18° (Kodis 1898), an egg of the silkworm to —18° or —24° (Pigorini 1931). Likewise Rein (1908) gives a full account of numerous experiments, from which it is evident that plants whose cell sap shows an almost equal osmotic pressure, manifest considerable differences in frost resistance. The following table is taken from Rein:

TABLE LIV

Species	Osmotic pressure of the sap, atmospheres	Death occurred at °C
Nepenthes Curtisii	6.30	— 2.00
Begonia	6.30	— 2.26
Helodea canadensis	5.60	— 4.60
Secale cereale	6.65	— 7.80
Spirogyra sp.	5.95	—14.20
Sphagnum acutifolium	5.95	—15.80
Hedera helix	6.65	—23.30
Oscillaria Froehlichii	5.60	—65.00

2. Factors favouring supercooling

The state of supercooling is favored by the following circumstances:

(*a*) *Dry surfaces*

Ice formation proceeds from crystallization centres which on their turn are formed on wet surfaces. Therefore when the surface is dry or coated with material insoluble in water (fat, wax), the system may be easily supercooled. Thus cut potato tubers freeze at —1°, while entire tubers may be cooled to —3° or to —4° without injury. Kodis (1898) saw that a frog muscle, wrapped in dry cotton-wool, could be greatly supercooled.

(*b*) *Capillarity*

In capillary spaces water may be frozen only at temperatures below 0° C, and a relationship exists between the size of the space and the degree of supercooling (Mousson 1858, Dufour 1861, Müller-Thurgau 1880, Bühler 1905). Morrison (1924), Bahrmann (1932) and others have so explained the supercooling in nerve, in bacteria, higher plants, etc.

(*c*) *Bound water*

Water may be supercooled similarly when it is bound in hydrophilic colloids, and the degree of possible supercooling depends on the force with which the water is held (H. W. Fischer 1911). This force increases with the content of such colloids, among which especially the pentosans seem to play an important part in the frost hardiness of plants (Hooker 1920, Rosa 1921*a*, *b*, R. Newton 1923—1924). Besides, the frost resistance will increase with decreasing water content, as it is known that such colloids are less hydrophilic when their water content is great. No doubt this is the chief cause of the high frost resistance of many spores, cysts, dry seeds and analogous formations (Davenport 1897—1899, Rahm 1920, Tomkins 1929). Of late the same principle has been applied to higher plants (Hooker 1920, Rosa 1921*a*, *b*, Strausbaugh 1921, R. Newton 1923—1924, B. S. Meyer 1928, Novikov 1928, Nilsson-Leissner 1929, Bobko and Popowa 1929, Busse and Burnham 1930) and to insects (Payne 1926*a*, *b*, 1927, 1928, Chapman 1928, Holmquist 1928*a*, W. Robinson 1928, 1929, Sacharov 1930). It has now been sufficiently demonstrated that the frost-hardiness of plants and of insects is associated with the hydrophilic power of their tissues. Dehydration in hypertonic media seems to increase the hardiness in certain plant cells (Fukuda 1932).

(*d*) *Protection against shock*

The protoplasm of living bodies is particularly well protected against mechanical shock which might cause its sudden freezing in an supercooled state (Pütter 1927; see also Kodis 1898).

It is important to note that the ability to assume supercooled state seems in certain cases to be intimately connected with the living condition of cells. F. J. Lewis (1920) has found that leaves of *Pirola rotundifolia* freeze at about —32° when living, but at —3.1° to —3.5° when dead. On the contrary Kodis (1898) pointed out that the tissues of warm-blooded animals can be progressively cooled below their freezing point, though they had already been killed by cold at a temperature above 0°.

3. Limits of frost resistance

In considering that supercooling depends on diverse factors, it is not always easy to determine the true limit to which a given organism may be cooled without dying. Moreover the length of

exposure seems to play an important part (see VIII. 6). It is thus not surprising that various authors have often found a different degree of frost resistance in one and the same organism. It would be outside the scope of this monograph to tabulate here the numerous data now available. Many results may be found in the following papers and monographs: DAVENPORT (1897), PFEFFER (1901), H. W. FISCHER (1911), KYLIN (algae, 1917), BRITTON (fishes, 1924), PÜTTER (1927), TIGERSTEDT (1910—14).

It may suffice here to point out some general features of frost resistance.

It is well known that certain organisms in a dry state, like grains, spores, cysts, etc., may withstand exposure to temperatures of —100° or more, even to temperatures approaching the absolute zero:

TABLE LV

System	Resisted t°	Resisted for	Authority
Dry seeds . . .	liquid air	> 3 weeks	P. BECQUEREL 1925
Dry seeds . . .	liquid hydrogen	>77 hours	P. BECQUEREL 1925
Dry seeds . . .	—250°	>1 hour	THISELTON-DYER 1899
Dry seeds . . .	—180° to —190°	>100 hours	H. T. BROWN and ESCOMBE 1898[1])
Pollen grains . .	—271°	several hours	P. BECQUEREL 1929
Spores of ferns .	—270°	6 hours	P. BECQUEREL 1930
Spores of mosses.	—271°	1 hour	P. BECQUEREL 1932*a*
Dry seeds of leguminous plants .	—190°	175 days	BUSSE 1930
Dried seedlings .	—271°	several hours	BECQUEREL 1932*b*
Eggs of certain insects	—190°	3½ hours	RAHM 1921
Dry nematodes and tartigrades	—190°	>20 months	RAHM 1924
	—272°	several hours	RAHM 1923
	—253°	>45 minutes	RAHM 1924

A high frost resistance has also been reported by many investigators for certain protozoa, algae and lower fungi, even in an active state:

1) This paper contains the earlier bibliography on the resistance of dry seeds to temperatures of liquid gases.

TABLE LVI

Species	Resisted	Authority
Putrefactive bacteria	—182° (1 hour)	Dewar and McKendrick 1892
Bacteria (various)	—70° to —120° (6 days)	Pictet 1884
Streptococcus	—18° (> 6 weeks)	Citovicz 1928
Luminous bacteria	—20°	Morrison 1924
Luminous bacteria	—182° to —190° (>20 hours)	Macfayden 1900
Bacteria (various, including luminous)	—190° (>6 months)	Macfayden 1902
Saccharomyces (sp.)	—190° (>7 days)	Macfayden and Rowland 1900*a*
Bacteria and saccharomycetes	—252° (>10 days)	Macfayden and Rowland 1900*b*
Beggiatioa alba	—47°	Rein 1908
Mucor mucedo	—70° to —110° (several hours)	Chodat 1896
Moulds and fungi from humus	— 70° (8 days) —183° (>13 hours)	Kärcher 1931
Chlorella	—182° (1 hour)	Warburg 1919
Trypanosomes	—190° (up to 2 hours)	De Jong 1923
Dunaliella viridis	—35°	Baas-Becking 1930

It is most probable that such a high degree of cold resistance is due chiefly to the small size (capillarity) of the cells, as suggested by Morrison (1924). Yet many primitive organisms seem capable of being supercooled even without regard to the size of their cells. The full list of data published by Rein (1908) shows that among plants many lower cryptogams are highly frost-resistant.

Likewise many terrestrial insects are particularly well adapted to withstand temperatures as low as several tens of degrees below 0° (see *e. g.* Davenport 1897—1899, Bachmetjew 1899—1901, Cameron and Brownlee 1914, Rahm 1921, 1924, Faes

and Staehlin 1921, Duval and Portier 1922, Payne 1926—1929, W. Robinson 1929, Sacharov 1930).

Tissues of homoiothermic organisms obviously cannot resist very low temperatures, yet there is now some information at hand that they, too, can be supercooled, even though not to such a degree as the cells of poikolotherms:

TABLE LVII

Tissue	Resisted t°	Resisted for	Authority
Spermatozoa of man	—6° to —17°	—	Davenport 1897—1899
Chick embryo .	—7°	a short time	Pictet (Kaestner 1895)
Chick embryo .	—4° to —6°	3—7 hours	Lipschütz and Illanes 1929
Explants from rat embryo. . . .	—5°	5 days	Simonin 1931
Explants from chick embryo .	—10° to —25°	3—33 hours	Bucciante 1929*b*, 1931
Mammalian muscles . . .	—7° to —10°	(begin to freeze)	Bottazzi 1920,1925

4. Hypothetical causes of death by freezing

The question why cells die on freezing might appear very simple, yet it is far from being so. On the contrary there is still much controversy on this point and it seems that the mechanism of death by freezing is not the same in every instance. Let us summarize in the form of a scheme the opinions which have been hitherto put forward with regard to the mechanism of frost-injury:

I. Ice forms in the cells, and the cell-wall bursts by the dilation of the freezing water. This, one of the earliest opinions, was definitely abandoned when Göppert (1830) and others could not detect any traces of bursting of cells in plants on freezing (see also Morren 1853, Sachs 1860*b*).

II. Ice formation is harmless in itself, but the cell is destroyed on thawing, as was first supposed by Sachs (1860*b*) for plants

and by Hoppe-Seyler (1877) for animal tissues. Hoppe-Seyler suggested that the tissue on thawing is suddenly flooded by distilled water and thus injured. This hypothesis was criticized by some botanists, especially by Goppert (1871) and by Bartetzko (1910), yet it seems now tolerably certain that under special conditions of temperature many plants, which can withstand freezing, are killed by a rapid thawing, as has been more recently found by Ockermann (1919, quoted by Novikov 1928), Akerman (1927), Novikov (1928), by D. C. Smith and Bressman (1930), W. S. Iljin (1933, Protopl. 20, p. 105) and Fukuda (1932).

III. Ice is formed around the cells, which are thus damaged from without, even when no ice is produced inside. As to the ultimate cause of death by such extraneous ice formation, which had been supposed by Sachs (1862) and Prillieux (1869) and was observed directly by Molisch (1897) and other botanists, three different mechanisms have been suggested:

(*a*) The cell is compressed mechanically and thus destroyed (Maximov 1914, 1929, Lepeschkin 1924, Rahm 1924, W. S. Iljin 1927).

(*b*) The forming ice withdraws water from the cell, the latter is desiccated (Müller-Thurgau 1880, 1886) and the concentration of substances dissolved in it or in the vacuole increases so as to become toxic or injurious to the colloidal state of the living matter (Gorke 1906, Lidforss 1907, H. W. Fischer 1911, Maximov 1914). On the other hand, Molisch (1897) thought that the dessication of the cell destroys its fine architecture. Rahm (1924) suggested the same mechanism in certain animals.

(*c*) Ice forming outside the cell destroys the protoplasmic surface-layer, which is the least resistant part of the cell. This hypothesis, which has been put forward by Maximov (1912), has not found much support (see Akerman 1927, Payne 1928).

The idea that death by freezing is nothing else than a death by drought has gained much popularity especially among botanists (besides those mentioned above see *e. g.* Matruchot and Molliard 1902, Wiegand 1906, Kylin 1917, Schander and Schaffnit 1919), yet it soon became evident that this is not the sole agent by which cells are killed when subjected to

frost, as first pointed out by Pfeffer (1904), Mez (1905), Irmscher (1912) and Czapek (1913). On the other hand, Bartetzko (1910) saw that certain moulds may die in an supercooled condition without any formation of ice. Besides, many organisms are known to withstand ice formation in their bodies without damage (see VIII. 5).

IV. Ice is produced in the interior of the cell, with a simultaneous ice formation around the cell. This again may involve a double source of injury, *viz.*, a mechanical destruction of the cell interior by ice crystals, or a frost coagulation of protoplasm, analogous to that of colloids. The latter of these mechanisms was admitted by Sachs (1860*b*), who, too, examined experimentally the coagulation of white of egg by freezing. Other similar investigations of colloids on the same lines were undertaken by Molisch (1897) and Lottermoser (1908). More recently Stiles (1930*a*) has expressed similar ideas.

V. Death occurs without ice formation by the direct action of cold, when the temperature falls beneath a specific minimum. Mez (1905) was the protagonist of this view. He suggested that in plants which resist ice formation, the ice rather plays a protective part, because it prevents, by its heat production and by its low thermal conductivity, a too rapid approach to the minimum. The experiments of Bartetzko, quoted above, lend support to the hypothesis of Mez. Similarly Rahm (1924) believes that ice protects certain insects against rapid frost-injury by its low thermal conductivity.

It is obvious that none of these mechanisms can be regarded as the universal cause of death by frost, but that several different mechanisms are often responsible for the frost-injury in various systems, and in one and the same system under unlike conditions. Sachs (1860*b*) suggested at least three distinct causes of damage by frost and similarly Pfeffer (1904), Mez (1905), Maximov (1914), R. Newton (1923), Lepeschkin (1924), Höber (1924), Åkerman (1927), Pütter (1927) and others admit various mechanisms causing death. In the first place it is clear that there is a difference between animal and vegetable cells, *viz.*, that the vacuole of the plant cell sets up special complications in the ice-formation. For the rest, the reader is referred to more detailed reviews by H. W. Fischer (1911), Åkerman (1927),

W. S. Iljin (1927), Nilsson-Leissner (1929) and Maximov (1929).

The most important changes which have been found to take place in freezing will be discussed in the next paragraph.

5. Changes produced by frost in the cell

Molisch (1897) observed that when a cell (*Spirogyra*, *Amoeba*) is cooled below the freezing point of water, ice crystals are first formed around its surface, and water is withdrawn from the interior. There is a considerable shrinkage of the cell. The vacuole of plant cells shrinks to such a degree as to assume the form of fine canalicules. When the cell-sap contains dyes, the vacuole appears as a thin coloured thread, or is reduced to small drops, intensely coloured (Maximov 1914). Considerable shrinking takes place similarly in *Amoeba* (Molisch) and in *Stentor* (Greeley 1902*a*) when frozen. A more detailed discussion of the literature on this subject is to be found in Akerman (1927). Many plant cells die when ice is once formed around them, but many others recover on thawing, as was known already to Göppert (1830), Sachs (1860*b*); (see further Schander and Schaffnit 1919, Warburg 1919, Zacharowa 1925, Akerman 1927, Maximov 1929, Stiles 1930*a*).

Similarly it is admitted that certain animals withstand ice formation inside their bodies (see especially Pictet 1893*a*, *b*, Bachmetjew 1901, Brunow 1912, Pütter 1927), yet the majority of investigators deny this possibility (Pouchet 1866, Cameron and Brownlee 1914, Weigmann 1929, etc.) Besides, it may happen that ice is formed without injury, but the organism is killed on further cooling, when its specific minimum is reached. Such a case has been reported by Brunow (1912). A frog muscle freezes completely at —4.06° and recovers after thawing, but is killed when cooled in the frozen state to —4.1° or —4.2°. It must be supposed that frost-resistant plants are killed similarly when their temperature decreases below a certain degree, as suggested by Mez (see VIII. 4).

When the cooling is rapid enough, ice may appear also in the interior of the cell (Molisch 1897). There is no satisfactory evidence that a cell would recover after ice had been formed in

it, yet such a possibility is admitted by Pütter (1927) and Stiles (1930*a*).

Consequent upon freezing, certain structural changes in the protoplasm have been reported. In the staminal hairs of *Tradescantia*, Kühne (1864, 1869) saw the protoplasm separate into several clumps, when cooled to —14°. The change was quickly reversible on warming. A similar observation was made by Klemm (1895). Yet this author emphasized that many objects die on freezing without any noticeable change of protoplasmic structure. In other cases Klemm described the appearance of granules in the cytoplasm. Naegeli (1860), Molisch (1897), Matruchot and Molliard (1902) found cytoplasmic vacuolisation to be a typical effect of freezing. Matruchot and Molliard saw that vacuoles formed also in the nucleus. Payne (1930) observed a general destruction of protoplasm in various animal tissues after freezing. But again some tissues died without showing any appreciable microscopical change.

As to the instances in which death occurs by the direct action of cold without formation of ice, there is no information at hand as to whether the protoplasm is modified in some characteristic way. Neither is it easy to say to what degree such cases are comparable with the phenomena of chilling, chill-coma and death by chilling, which are discussed in full in Chapter IX. At any rate, the protoplasmic changes described hitherto as due to the action of frost are not typical, and the same modifications of the protoplasmic structure have often been found to accompany injury by heat (see X. 6, 13) and by other agents.

Besides these morphological phenomena, the action of temperatures below 0° involves a change in the chemical properties of living systems. It was known to Kühne (1864—1869) that a liquid rich in proteins separates from a frozen muscle on thawing. Hermann (1871), who repeated these experiments, stated that the liquid is acid. It is now known that this is due to the accumulation of lactic acid at the time of thawing (Fletcher 1913; see further bibliography in Bottazzi 1925).

R. B. Harvey (1918) stated that the liquid expressed from frozen leaves has a lower pH-value than normal. He believes that the increased acidity favours the denaturation of proteins by frost.

Heilbronn (1929) has found that leaves of *Polypodium vulgare*, when killed by an exposure to −17°, produce prussic acid. This phenomenon however is not a specific effect of freezing, but accompanies death by mechanical injury as well. Neither are the chemical changes which take place in the muscle an exclusive sign of frost injury, because lactic acid is formed by many other kinds of injury in muscle.

Dexter, Tottingham and Graber (1930) find that the concentration of electrolytes in the liquid expressed from plant tissues increases when the plant has been previously frozen, and so likewise does the electric conductivity of the liquid and that of the tissue (1931). This observation has been confirmed by S. Ivanov (1931), who finds that the concentration of electrolytes shows a marked increase even before any morphological change can be observed. The phenomenon is at first reversible, and gains in intensity progressively with the length of exposure until death occurs, after which moment there is no further increase in conductivity. Ivanov assumes that electrolytes separate from proteins under the action of low temperatures.

6. Time-factor in the effects of frost

It has long been appreciated that time is an important element in the action of frost (Göppert 1830. The earlier data have been reviewed by Davenport 1897–1899, to whose book the reader is referred). The general conclusion is that at a given temperature the effect increases with the time of exposure, or that at a higher temperature a longer exposure is necessary to produce a given effect than at a lower temperature. Let us quote a few instances from the more recent and perhaps more accurate work.

According to Quajat (quoted from Pigorini 1931) the embryos of the silkworm are killed by −10° in 49 days, by −24° in 30 days. Rahm (1924) observed that certain insects, which withstand a short exposure to −15°, die even at −1° when the exposure is long.

G. Smith (1920) found that the rate of killing by frost in the case of *Staphylococcus* depended on temperature and time, as follows:

TABLE LVIII

Temperature	90% killed after (days)	All killed after (days)
+8 to +10°	15—31	>60
— 1°	8—9	19
— 6°	5—6	13
—11°	3—4	9

Hogetop (1930) has examined the germinating power of potato tubers as influenced by frost, and has arrived at the following figures:

TABLE LIX

Temperature	Slight injury	Strong injury	Death
—4°	6 hrs.	8 hrs.	—
—6°	4½ hrs.	—	8 hrs.
—9°	—	2 hrs.	3½ hrs.

The fertilized eggs of *Ascaris* withstand, according to Zawadowski (1926), the following temperatures and exposures:

TABLE LX.

Temperature	Exposure	Remarks
+4 to +5°	5 months	Normal development after subsequent warming
— 5°	1 month	Normal development after subsequent warming
—20°	14 days	50% degenerated

After two months of exposure to —24°, there is still a possibility of development on warming, yet the embryos finally die. A similar degree of injury is produced by an exposure to —10° for a year and three months.

Similarly it is known that explants from homoiothermic organisms resist frost for a time which varies inversely with the temperature (Bucciante 1929*b*, 1931*a*, *b*, Simonin 1931; see also Craciun 1931). The following data are abstracted from a paper of Bucciante (1931*a*) on explants from the chick embryo:

TABLE LXI

Organ (Tissue)	Survived at 0°	—5°	—10°	—14°	—25°
	days	hours			
Epidermis	32	40	37	33	3
Cornea	28	28	26	11	3
Heart	10	18	16	11	—
Muscle	22	28	26	16	—
Brain	10	18	15	—	—
Leucocytes	5	12	8	—	—

Thus the rule is now sufficiently established that the exposure which is necessary to produce a given frost effect decreases with decreasing temperature. Yet this is not always necesarily so. DE JONG (1923) found in trypanosomes which were subjected to temperatures ranging from —20° to —191° that in certain cases death by frost occurred in agreement with the general rule, but in the majority of species examined, the temperatures of —20° and —30° were more deleterious than that of —190°. For instance death occurred in *Trypanosoma lewisi* at —20° and —30° in 80 and 60 minutes respectively, whereas at —190° two hours were necessary for killing. In *T. equiperdum* an exposure of $3\frac{1}{4}$ hours was sufficient to produce death at —20°, while at —191° virulence was conserved for more than 20 days. Similarly *T. venezuelense* and *T. brucei* were quickly injured at —20°, but for a long time resisted a temperature of —190°. DE JONG quotes these experiments, which were performed by his collaborator ZANDBERGEN, without any explanation. PÜTTER (1927), who analyzed them, believed that death was due to an accumulation of toxic metabolic products, which formed more quickly at higher temperatures. Yet it seems more natural to explain the phenomenon on a physical basis. TAMMANN (see H. S. TAYLOR 1931, p. 1035) established the fact that the rate of crystallisation in supercooled solutions depends on temperature in such a way that there is a maximum velocity at a definite temperature, above and below which the rate is less. The rate of crystallisation according to temperature thus gives a curve with an optimum. If it may be assumed that in the experiments of ZANDBERGEN death was caused by crystallisation of ice in supercooled protoplasm, the temperature of —20°

may be conceived as the optimum for the velocity of crystallisation.

In this connection we may mention the part played by rapidity of cooling. Molisch (1897) showed that on rapid cooling the effect of frost on plants is more injurious than when the tissue is cooled gradually, and that ice is formed only around the cells when the cooling is progressive, while ice may appear also in the interior of the cell when the cooling is sudden. Likewise Pictet (1893*b*) saw that the eggs of the frog withstood -60^0 only when the cooling was slow, and Rahm (1924) observed the same in water-beetles. Akerman (1927) and Pütter (1927) sum up our present knowledge in concluding that a slow cooling favours the state of supercooling, and *vice versa*.

As was recently pointed out by Stiles (1930*a*), the size of ice crystals is smaller, the more rapid their formation is, and consequently the degree of injury by freezing should be greater when the freezing process is slower. A rapid cooling should therefore be less injurious than a progressive one. This conclusion of Stiles, though contrary to what has been hitherto known for living systems, seems to be in agreement with certain facts which concern the freezing of chlorophyll and of muscular tissue, but the author himself admits that even in gelatin sols the process is not so simple. He quotes, in support of his idea, the results of Kühne (1864) and of Molisch (1897) on the freezing of staminal hairs of *Tradescantia* and he points out that Kühne, who cooled his objects quickly, saw a recovery on thawing, while Molisch, who changed the temperature only slowly, found the cells dead. It is however highly questionable whether such a comparison is justified, considering that Kühne and Molisch worked with two different species of *Tradescantia* and under different experimental conditions.

When a system in a supercooled state has once reached the temperature of its environment, the length of exposure may still have an effect, because the probability that disturbances will take place increases with time.

As yet nobody has seriously attempted to describe by a formula the relationship between time and temperature below zero, or the relationship of velocity of damage to such temperatures. The data available at present are indeed too few to allow of a mathematical treatment. (See Meissner 1913, Rahm 1924).

7. Internal factors in the effects of frost

The great variability of living systems in their resistance to frost justifies the question whether the effects of temperatures below 0^0 depend on some causes within the organisms themselves.

First of all, various organisms or tissues may differ in their ability to become supercooled, and their resistance to frost may thus be unlike because of unlike factors which favour supercooling, and which have been enumerated above (VIII.2). Secondly, the organisms may differ in their chemical properties. These have been the subject of much study chiefly in connection with the frost resistance of plants. It would be out of place to discuss here in full the extensive work hitherto published, especially in view of the fact that there is little unanimity among the investigators. May it suffice to say here that all the main chemical constituents of protoplasm, *i. e.* sugars, fatty substances, proteins and salts are each considered by various workers as the sole cause of frost-resistance and of adaptation to frost.

The idea that the presence of sugars in the cells of plants increases their resistance to frost was introduced by Lidforss (1907), who based his belief on the well known fact that the amount of sugar increases considerably in many plants when winter approaches, the reserves of starch being split into sugar. We have already had occasion to discuss these and similar changes above (V.7). Lidforss thought that sugars protect the proteins from being coagulated by freezing. He also found that a solution of protein was less easily denatured by frost after sugar had been added, than without it. Maximov (1908, 1912, 1914, 1929), Schaffnit (1911), Schaffnit and Wilhelm (1933), Iraklinow (1912) and others added to the evidence on the protective action of sugars, but Maximov found at the same time that other simple substances, *e. g.* glycerine, alcohols and certain salts, exert a similar action. Likewise Pantanelli (1920) stated that certain salts modify resistance to freezing in roots. Detailed reviews of this problem are those of Akerman (1927) and of Maximov (1929; compare also Nilsson-Leissner 1929). It is not yet clear which property is the protective agent in the case of the substances in question. Akerman is inclined to believe that the mechanism is physical, the power of the cell to retain water in a liquid state being dependent on the amount of osmotically active substances dissolved. In reality it has long been supposed

by many botanists that the resistance of plants to freezing is more or less closely associated with the osmotic concentration of the cellular contents (see VIII. 1: Rein 1908, H. W. Fischer 1911, Gortner and Harris 1914). Pütter (1927) thinks that the osmotic concentration is the chief cause of frost resistance in general, but that its effect is often hidden because of the phenomena of supercooling.

Zacharowa (1925) found that the resistance of plant tissue to freezing is connected with a higher pH value. She thinks that acidity makes the proteins less stable and thus easily coagulable by freezing.

The protective action of fats was assumed by Mez (1905). There is in fact a notable accumulation of fat in many plants at the approach of cold weather (Iraklinow 1912).

Schaffnit (1911) thought that proteins together with sugars play an essential part in determining frost-hardiness. According to him, protoplasm produces simpler and more stable proteins, the lower the temperature. This however is still an open question, though actually it is known that the properties of proteins vary with the temperature at which they have been formed (see V. 7).

The following biological factors bear upon the response of organisms to temperatures below zero:

(*a*) *Adaptation.*

When plants or their parts are grown or kept at lower temperatures, they usually withstand frost better than when cultivated or stored at higher temperatures. Göppert (1830), Müller-Thurgau (1882), Apelt (1907), Rein (1908), Schaffnit (1911) and others produced sufficient evidence as to the ability of plant tissues to adapt themselves to cold. Yet the limits of their adaptation to frost are rather narrow, so that when the temperatures of cultivation or of storage differ by a matter of twenty degrees, the resulting difference in the temperature at which the plants die is only one or two degrees, as was shown by Apelt (1907) and by Rein (1908) for several plants. Apelt examined the development of hardiness in potato tubers, and arrived at the following figures:

TABLE LXII

Exposure to cold	Frost death-point lowered
4 days	0.07°
15 days	0.34°
28 days	0.68°

From the analogous data of Rein (1908) it may be concluded that the process is slow in the first two days, then more rapid in the ten following days and slow again towards the end.

The majority of investigators are inclined to explain the frost adaptation of plant tissues on the basis of sugar accumulation, while Schaffnit (1911) thought that the chief protective agents are sugars together with proteins. In the light of the more recent investigations of Rosa, R. Newton and others, whose work has been discussed above (p. 120), it seems very likely that the hydrophilic colloids in the cells or in the cell-walls are the most efficient agents in this respect.

(*b*) *Age.*

Organisms and organs of different age often show unlike resistance to freezing. The chief data now at hand are summarised in the table LXIII (p. 135). It is evident that age does not modify resistance to frost in a uniform way. The conclusions of some investigators that the resistance to freezing increases (J. Loeb 1906) or decreases (H. W. Fischer 1911) universally with advancing age, therefore are not sufficiently founded. Neither is it always known which biochemical or colloidal factors are responsible for such age differences. H. W. Fischer (1911) suggested that in young tissues water is retained by a greater force and that their cells thus are more resistant. Hooker (1920) supposes that a high pentosan content is the cause of greater resistance in adult plant tissues, as compared with the younger parts. Bartetzko (1910) pointed out that the older filaments of certain moulds are more resistant in spite of their larger diameter and lower osmotic concentration.

TABLE LXIII

(Differences in frost resistance according to age)

Organism	Stages examined	More sensitive stage	Authority
Yeast cells	—	old	Schumacher 1874
Enteromorpha intestinalis	cells of various age	old	Dehne (*cf.* H. W. Fischer 1911)
Moulds	hyphs of various age	young	Bartetzko 1910
Bacteriophage . .	—	old	D'Hérelle 1926
Laminaria	—	young	Kylin 1917
Seeds	storage of varied length	old	Estreicher-Kiersnowska 1915
Hairs of *Tradescantia*	cells of different age	young	Klemm 1895
Various plants . .	leaves	old	Göppert (*cf.* H. W. Fischer 1911)
Various plants . .	leaves in buds and developed leaves	old	Noll 1885
Allium cepa. . . .	bulb scales	old	Rein 1908
Shoots of potato .	apex and base	young	Apelt 1907
Shoots of apple tree	apex and base	young	Hooker 1920
Sequoia and other trees	different parts	young	Huber 1929
Wheat	plants of various degrees of development	seedlings	J. F. Martin 1932
Dendroctonus brevicornis	eggs, larvae, pupae	old	J. M. Miller 1931
Anthonomus grandis	eggs, larvae, pupae	young	Pierce 1916
Pulmonate molluscs	embryos, adults	old	Roedel 1886

(*c*) *Different tissues and cells.*

Haberlandt (1879) knew that there are certain differences in frost resistance in chloroplasts of various cells, those of the guard cells being more resistant than chloroplasts of the mesophyllic cells. According to Molisch (1897), the stomatal cells themselves are less sensitive than other cells of the same plant. According to Zacharowa (1925), the root-hairs and the cortex

are more sensitive than the meristematic tissue. Roots seem to be less resistant than the upper parts of the plant (Tumanow and Borodin 1930), and even the top and the base of the root may show an unlike degree of hardiness (Dexter, Tottingham and Graber 1932).

Similarly the tissues of animals are not all equally sensitive to frost. Fraser Harris (1910) showed that the nervous tissue and the heart of the frog are more easily killed by freezing than certain muscles. Weigmann (1929, 1930) who summarized the earlier work, concludes that the peripheral nerves of the frog are very resistant, but he found that the central nervous tissue is rapidly destroyed by freezing, as was also suggested by Cameron and Brownlee (1914). Simonin (1931) states that the explanted nervous tissue is the least resistant of all tissues of various mammalian embryos, and believes that the high degree of differentiation is the cause. Analogous differences between explants of various tissues have been established by Bucciante (1929—1931), as pointed out above (VIII. 6, p. 130). According to his results, the nervous cells (mesencephalon) are among the most sensitive.

(*d*) *Living and dead tissue.*

We have seen above (p. 120) that the ability of certain plant tissues to become supercooled disappears after death (F. J. Lewis 1920). Besides that, it is known that there is a difference between the live and dead muscle in the progression of changes which take place during freezing (H. W. Fischer 1911). Yet Kodis (1898) succeeded in supercooling dead tissue of homoiotherms.

CHAPTER IX

CHILLING, CHILL-COMA AND DEATH BY CHILLING

1. General and historical remarks

As early as 1778 it was known to BIERKANDER (quoted by SACHS 1860*b*) that certain plants are unable to withstand for long an exposure to temperatures lying just above zero. SACHS (1860*b*) saw that the temperature of $+5^0$ was injurious to tobacco and other plants of similar ecological relationship and he thought that a disproportion between the intake and output of water was the cause of injury.

Analogous effects of temperatures above the freezing point of water were also known to animal physiologists. ROSSBACH (1872) described an arrest of ciliary movement in protozoans kept for a short time slightly above 0^0. HORWATH (1876), basing his studies on the commonly known fact that mammals die when their body temperature falls below a certain point, examined particularly the effect of cold on the motor nerves and was first to describe what is now called "anaesthesia by cold". ENGELMANN (1877), SEMPER (1880), DAVENPORT (1897—1899) collected most of the earlier data on this subject.

Yet the problem has been approached from still another angle. Guided by practical interests, some botanists determined what they called the "specific zero" of vegetation, *i. e.* the temperature below which a given plant cannot grow, thus giving rise to the first attempts at a mathematical treatment of the velocity of vital processes as influenced by temperature. We have already quoted above (II. 2) the names of P. DE CANDOLLE, BOUSSINGAULT and BURCKHARDT in this connection. It soon became clear that the "specific thermal zero" or "threshold of temperature" does not always coincide with 0^0 C, but that it is

often situated above it. The term "chill coma" was first used, it seems, by SEMPER (1880). ROSSBACH (1872—1874) and DAVENPORT (1897—1899) denoted the same physiological state as "temporary rigor by cold".

It is obvious that the mechanism which produces this state is widely different from that of freezing, *i. e.*, formation of ice crystals in the cell or in its close vicinity. This, too, was recognized already by the first students of chilling phenomena, especially by botanists GÖPPERT (1830) and SACHS (1860*b*).

Yet there seems to be a close resemblance, if not identity, between the action of temperatures above 0°, which chilling occurs, and that of temperatures below 0°, when vital action ceases, temporarily or permanently, without formation of ice. Such cases have been dealt with in the preceding chapter (VIII. 4). The most typical is that described by BARTETZKO (1910), who found that moulds, kept in a supercooled state, still die, even without freezing. MOLISCH (1897), MEZ (1905) and BARTETZKO (1910) consider such instances as being analogous to chilling at temperatures situated above 0°. The only difference to be pointed out is that injury or death takes place more quickly when the organism is chilled at temperatures below 0° than at those lying above it (ÅKERMAN 1927).

To avoid any confusion, we shall use the term "biological zero" to denote the temperature at which a given protoplasmic action is arrested by cold without formation of ice. It is clear that in cases where the biological zero lies much below 0°, it would be possible to determine it only when the organism could be supercooled for a sufficient time and to a sufficient degree. We shall reserve the term "temperature minimum" to denote the temperature at which an organism still can, or just cannot, live on in an active state. In the present monograph, this ecological minimum will not be dealt with, though it obviously may often depend on the biological zero of one or another protoplasmic activity. Thus defined, the biological experimental zero is identical with the mathematical zero, as discussed in Chapter II (see II. 2, II. 3, II. 7, II. 8).

2. Biological zero of various vital activities

It may be useful to give here a table relating to various biological phenomena and to different species. It is, however,

to be noted that the biological zero varies sometimes according to internal and external factors, which are to be discussed separately (IX. 6, IX. 7), so that the numbers tabulated here do not have absolute value. Where possible, the factors which might bear upon the numerical value of the data are mentioned in the table.

TABLE LXIV

Biological zero (*i. e.* temperature at which vital processes are arrested by cold) of various activities

Species	Biological zero	Remark	Authority
A. Protoplasmic movement			
Nitella syncarpa . .	0°		Naegeli 1860
Chara foetida . . .	0°		Velten 1876
Vallisneria spiralis .	0°		Velten 1876
Helodea canadensis .	0°		Velten 1876
Cucurbita pepo, hairs	11—12°	only traces of movement	Sachs 1864*a*
Caulerpa prolifera .	6°		Dostál 1929
Phaseolus, leaves .	6°		Curtis 1929
Marine amoebae . .	—3.1°		Pantin 1924
Leucocytes of frog .	2—5°		Ueki 1928
Leucocytes of horse	15°		De Haan 1922
Leucocytes of rabbit	10—11°		Ono 1928
Leucocytes of rabbit	10°		Sugiyama and Mori 1927
Leucocytes of man .	12°	forms pseudopodia without moving	Comandon 1919
Leucocytes of man .	10—11°		Ueki 1928
B. Ciliary movement			
Ulothrix zonata, spores	0°		Strasburger 1878
Haematococcus lacustris, spores . . .	$<0°$	can move in thawing ice	Strasburger 1878
Botrydium granulatum, spores . .	6°		Strasburger 1878
Euglena viridis . . .	5—6°	collected in summer	Schwarz 1884

Species	Biological zero	Remark	Authority
Euglena viridis . . .	$<0^0$	collected in winter	Adershold 1888
Stentor coeruleus . .	2^0		Greeley 1902*a*
Monas sp.	4^0		Greeley 1902*b*
Ciliates (various) . .	$2—3^0$		Rossbach 1872
Ciliates (various) . .	4^0		Schurmayer 1890
Mytilus edulis, epithelium . . .	$<0^0$	at 0^0 still beat	Gray 1923
Ox trachea, epithelium	15^0		Umeda 1929
C. Cellular multiplication			
Didymium nigripes .	$2—4^0$		Skupieński 1930
Staphylococcus pyogenes	$8—10^0$		G. Smith 1920
Ulothrix zonata, sporulation	$1—2^0$		Oltmanns 1923
Saccharomyces Saké .	9^0		Nishiwaki 1929
Bacillus thermoamylolyticus	$30—35^0$		Coolhaas 1928
Bacillus tuberculosis .	30^0		Jost 1913
Thermophilic bacteria	$20—35^0$		Mischustin 1927
Thermophilic bacteria	$37—40^0$		Muller 1928
Herpetomonas donovani	16^0		Christophers and authors 1926
Eggs of Echinoderms	-2^0 to -3^0		O. Hertwig 1890
Paracentrotus lividus, eggs	$5—8^0$	winter	Hörstadius 1925
Paracentrotus lividus, eggs	$9—16^0$	summer	Hörstadius 1925
Cyprinus carpio (maturation of reproductive elements) .	17^0		Hesse 1924
Myoblasts of chick embryo	24^0		Bucciante 1926
Myoblasts of chick embryo	21^0		Bucciante 1927*a*, *c*
Fibroblasts of chick embryo	$20—30^0$		Nemoto 1929

Species	Biological zero	Remark	Authority
		D. Development and growth	
Ceratium hirundinella	5°	excystation	Huber and Nipkow 1923
Fungi:			
Botrytis cinerea . . .	0°		W. Brown 1922
Sphaeropis malorum .	3—5°		W. Brown 1922
Fusarium (various species)	3—5°	growth of mycelium	Togashi 1931
Fusarium (various species)	5—10°	development of spores	Togashi 1931
Endothia parasitica .	7°		Stevens 1917 *a*, *b*
Acremoniella thermophila	27—30°		Curzi 1930
Higher plants:			
Hordeum vulgare . .	5°	roots	Sachs 1860 *a*
Triticum sativum . .	5°	roots	Sachs 1860 *a*
Phaseolus multiflorus	9.3°	roots	Sachs 1860 *a*
Zea mais	9.3°	roots	Sachs 1860 *a*
Cucurbita pepo . . .	13.5°	roots	Sachs 1860 *a*
Solanum tuberosum .	6°	shoots	Hogetop 1930
Scirpus kisoor . . .	22°	tropical	Bose 1918
Worms:			
Ascaris megalocephala	7°		Zawadowski 1926
Oxyuris	20°		Zawadowski and Schalinow 1929
Ancylostomum caninum	12—15°		McCoy 1930
Fasciola hepatica . .	12°		Mattes 1926
Insects:			
Calandra granaria .	9.5°		Bodenheimer 1927
Calandra oryzae . . .	13.1°		Bodenheimer 1927
Bombyx mori . . .	>9°		De Candolle 1865
Toxoptera graminea .	4.5°		Chapman 1928
Ephestia kühniella .	3—5°		Hase 1927
Deilephila euphorbiae	9°		Heller 1928

Species	Biological zero	Remark	Authority
Bucculatrix canadensiella	10—12°		Friend 1927
Popillia japonica . .	10°		D. Ludwig 1928*a*, 1930
Sphodromantis biocullata	15°		Przibram 1916
Lucilia sericata . . .	8—10°		Cousin 1929
Lucilia sericata . .	10°		Wardle 1930
Crustacea:			
Daphnia pulex . . .	10°		Tauson 1930
Moina macrocopa . .	3.2°		Terao and Tanaka 1928
Molluscs:			
Limnaea stagnalis .	12°		Semper 1880
Fishes:			
Gasterosteus aculeatus	6°		Leiner 1932
Pleuronectes platessa .	—2.4°		Haempel 1913
Pleuronectes flessus .	—1.8°		Haempel 1913
Gadus morrhua . .	—3.6°		Haempel 1913
Amphibians:			
Bufo lentiginosus . .	6°		Lillie and Knowlton 1898
Rana virescens . . .	3°		Lillie and Knowlton 1898
Rana fusca	5°		Kaestner 1895
Rana fusca	0°		O. Hertwig 1898
Birds:			
Chick embryo . . .	28°		Dareste 1891
Chick embryo . . .	21°		Kaestner 1895
Chick embryo . . .	20—21°		Edwards 1902
E. Metabolic processes			
Phascolus multiflorus	1—6°	transport of carbohydrates	Curtis 1929
Tropical plants. .	4—8°	production of O_2	Ewart 1896

Species	Biological zero	Remark	Authority
Lupinus	—2°	still a slight production of CO_2	Pütter 1927
Triticum vulgare . .	—2°		
Phaseolus multiflorus	8—10°	formation of chlorophyll	Sachs 1864*b*
Zea mais	13—14°	formation of chlorophyll	Sachs 1864*b*
Triticum ferrugineum	2°	formation of chlorophyll	Ljubimenko and Hubbenet 1930
Selaginella martensii .	10°	formation of chlorophyll	Schwarz 1930
Phaseolus	8°	restoration of nucleus after starvation	Maige 1923*a*
Urobacillus psychrophilus	>—2.5°	still decompose urea	Rubentschik 1925
Urosarcina psychrophila			
Solanum tuberosum .	3°	permeation of *Br*	F.C.Steward 1932
Gammarus chevreuxi.	10°	pigment formation in the eye	Ford and Huxley 1927

F. Muscular movement

Cassiopea xamachana	9.5—10.6°		E. N. Harvey 1911
Dog diaphragm . .	0° to 7°	contracture	Bottazzi 1920
Homoiotherms, smooth muscles .	15—20°		Bottazzi 1925

G. Heart beat

Rana sp.	0°to — 4°		Cyon 1866
Rana sp.	2°		Goudsmith 1929
Limulus polyphemus.	0° to —1°		Carlson 1906
Lacerta agilis, embryos	—2°		Andersen 1929*a*
Cat	16.5°		Martin (*cit.* Edwards 1902)
Cat	16.0°		Britton 1922
Rabbit	14—15°		Herlitzka 1905
Mammalian heart .	10—12°		Snyder 1913*a*
Mammalian heart .	17°		Tait 1922

Species	Biological zero	Remark	Authority
Explants from human embryonic heart	16—18°		Höfer 1931
Explants from chick embryonic heart	12°		Bucciante 1927 *b*
H. Excitability and nervous functions			
Mimosa pudica	14°		Wallace 1931
Prothesia, larvae	13°	phototropic response	J. Loeb 1890
Cassiopea xamachana	8.8—9.5°	nervous conduction	E. N. Harvey 1911
Cassiopea xamachana	14°	pulsations	E. N. Harvey 1911
Rana sp.	—7°	nervous conduction	Boycott 1902
Rana sp.	—2° to —10°	nervous conduction	Garten and Sulze 1913
Rana sp.	5—15°	nervous conduction	Howell and authors 1894
Rana hexadactyla	4.1°	activity of medullar centers	Garten and Sulze 1913
Rana hexadactyla	5—11°	action currents of nerves	Garten and Sulze 1913
Rana hexadactyla	0.25—1.0°	action currents of nerves	Garten and Sulze 1913
Alligator mississipiensis	3.5—8.5°	nervous conduction	Weigmann 1929
Cat	7°	nervous conduction	Boycott 1902
Cat	7°	spinal ganglion	Artom 1927
Cat	15°	sympathetic ganglion	Artom 1927
Cat	4—7°	nervous conduction, sympathetic	Artom 1927
Cat	16—34°	cerebral and spinal functions	Britton 1922
Cat	16—30°	cerebral and spinal functions	Simpson and Herring 1905
Rabbit	3.8—7.4°	nervous conduction	Alcock 1903

Species	Biological zero	Remark	Authority
Rabbit	7°	nervous conduction	Eve 1900
Rabbit	5—8°	excitability of motor fibres	Granberg and Hollander 1927
Rabbit	19—34°	cerebral and spinal functions	Winternitz 1905
Rabbit	19—26.5°	superior cervical ganglion	Eve 1900
Dog.	1—6°	nervous conduction, sciatic	Grützner 1878
Dog.	2—6°	nervous conduction, vagus	Grützner 1878
Dog.	5—8°	nervous conduction, sciatic	Wollman and Lecrenier 1907
Hedgehog	—1.4 to +6.4	nervous conduction, during hibernation	Alcock 1903
Pigeon	6.9—8.2°	nervous conduction	Alcock 1903

3. Death by chilling

When an organism is chilled for a short time, the arrest of its vital activities is usually reversible on subsequent warming. When the exposure is long enough, the changes produced by chilling are however irreversible, and death thus takes place. This was clear even to those authors who were the first to study the phenomena of chilling in plants and in animals (see IX. 1). It is known that in mammals death occurs when the body temperature falls below 14°—22°, according to the species and other circumstances. For details regarding mammals, the reader is referred to the following literature: Horwath (1876), Semper (1880), Bouchut (1883), Simpson (1902), Simpson and Herring (1905), Winternitz (1905), Lefèvre (1911), Pütter (1911), Laurent (1921), Britton (1922), Sinitzin (1923), Yamaguchi (1927), Tigerstedt (1910—14).

Death may likewise occur on chilling in poikilotherms, which are adapted to higher average temperatures. Certain reptiles are very easily killed by cold at relatively high temperatures,

as was recently confirmed by Weigmann (1929). This obviously applies to any thermophilic organisms, such as are met with in many animal and plant groups.

Certain ciliates die at about -3^0, as found by Rossbach (1872), fertilized eggs of *Artacia* may be killed at -4^0, when the exposure is sufficiently long, as recently confirmed by Payne (1930). Embryos of *Culex* often die when cooled to -5^0 (de Boissezon 1930), embryos of the worm *Spirorchis* are killed at 0^0 to $+2^0$ within 5 hours (Onorato and Stunkard 1931), certain cladocerans do not recover after a chilling to 3^0 or even to 10^0 (Terao and Tanaka 1928; L. A. Brown 1929*a*). Wood and Banta (1926) have succeeded in obtaining a thermophilic mutant of *Daphnia*, which died of cold at temperatures lower than 20^0, while ordinary species are active even at 1^0 (L. A. Brown 1929*a*). Other similar references may be found, *e. g.*, in Maurel and Lagriffe (1899), Pütter (1911, 1927), Weigmann (1929).

The majority of workers agree in ascribing death by chilling in homoiotherms and in higher animals to an irreversible injury of the central nervous system, whose protoplasm thus would be the most sensitive. We shall see below (IX. 6*c*) that the protoplasm of various regions of one and the same body or tissue often differ in their sensitiveness to chilling.

Death by chilling in plants has been studied by Bierkander (1778), Sachs (1860*b*), Ewart (1896), Molisch (1897), Pfeffer (1904) and other botanists. In general, exposures of several hours, days, or even weeks are necessary to produce death in plants.

4. Cellular changes accompanying chilling

For the understanding of what is the probable cause of chilling it is important to know what happens in the cell when it is chilled.

The most striking effect of chilling obviously is an arrest of the vital functions. Further an increase in viscosity has been demonstrated by several investigators to take place at temperatures which produce chilling. This point has been analysed in detail above (VII. 1). It is very probable that some other changes, which have been described as concomitants of chilling, are directly due to changes in the protoplasmic viscosity, *e. g.*, a broad and low fertilization cone in the eggs of the sea-urchin (O. Hertwig 1898), or petaloid pseudopodia in the amoebocytes of a starfish

(Fauré-Fremiet 1929). Ishikawa (1913) has studied the rounding up of an *Amoeba* on application of a lower temperature (slightly over 0°), a phenomenon which was first described by Engelmann and which occurs, under similar conditions, in other types of cells as well: in *Stentor* at +2° and in *Spirogyra* at 1°—2° (Greeley 1902*a*), in *Monas* at +4° (Greeley 1902*b*), in epidermal cells of higher plants at 0° to +2° (Åkerman 1915), in the ciliated spores of the phycomycete *Plasmopara viticola* at 0° (Arens 1929*b*), in the fibroblasts of the chick embryo at room temperature (Tannenberg 1930). All these changes are reversible when temperature is raised.

Under the action of cold, the cellular content is sometimes displaced in an atypical way. Dostál (1929) noticed a clustering of chloroplasts in *Caulerpa prolifera* on transfer to 1°—2° for 30—90 minutes. Payne (1930) states that in the eggs of certain marine animals (*Cumingia*, *Arbacia*) a stratification of their contents occurs at lower temperatures (0°—12°), a phenomenon similar to that taking place normally on centrifuging.

As regards the cytoplasmic ground substance, a reversible vacuolisation has been observed as a consequence of chilling by Nägeli (1860), Kühne (1869), Klemm (1895), in plant cells and by Greeley (1902*a*) in *Stentor*. In this respect there seems to be little difference between chilling and effects of frost without crystallization of ice. Besides, several observers saw that granules appeared in the cytoplasm when chilled. Fawcett (1921) observed such fine granules in the hyphae of certain parasitic fungi when cultivated at 7.5°, while at an ordinary temperature the protoplasm was limpid. It is not certain whether such structures are due to coagulative changes of protoplasmic colloids, or rather to a precipitation of substances which are insoluble at low temperature or which perhaps accumulate because of a decreased permeability. Yet Pantanelli (1919) saw an increased permeability in chilled vegetable tissue. de Moulin (1924) has observed that certain types of white blood cells and of glandular cells of warm-blooded animals, whose cytoplasm is normally limpid, formed granules when cooled to room temperature. Similar granulation however takes place in these and in other cells on application of various agents other than cold. O. Hartmann (1919*c*) has described a coarse granulation of the nucleus in the leaves of *Helodea*, cultivated in cold. M. M. Mack (1933) observed

retraction of nuclei in tissues of chilled turtles. These investigators however worked with fixed preparations.

Kaestner (1895) saw that the young chick embryo was less transparent, when the egg had been cooled, than at a normal temperature.

So far as our present knowledge goes, no specific change occurs in the cell on chilling. All the phenomena described hitherto as being due to the action of low temperatures above zero, may take place under the action of other factors as well. In fact there seems often to be little or no difference between the coarser changes which take place in plants on chilling and on freezing (Pantanelli 1918, Wartenberg 1929).

Many observers have noted that the mitotic process is altered at lower temperatures. O. Hertwig (1890) described a disappearance of the spindle in dividing eggs of sea urchin at 1^0—4^0. This has been recently confirmed by Vintemberger (1930) in the avian embryo. Němec (1899) found that the formation of the equatorial plate in the dividing plant cells was hindered at 4^0—6^0, while in the prophase and metaphase the process was but slightly affected. Hovasse (1923) observed that the telophase was greatly affected by cold. According to Rudloff and Schmidt (1932) the cell is the most sensitive in the anaphase. The migration of chromosomes may easily be slowed or arrested under such conditions, and abnormal cells may thus be formed. As a matter of fact, it is known from the work of Gerassimow (1901) on *Spirogyra* that at low temperatures giant or polyploid cells are produced. Atypical mitoses in cold have since been described by Sabline (1903), Bury (1913), P. Michaelis (1926), Shimotomai (1927), Bucciante (1927*b*, 1928), Spear (1928), Koshuchov (1928), Bleier (1930), Vintemberger (1930), Rudloff and Schmidt (1932), M. M. Mack (1933) and others. Gottschewski (1932) has recently obtained cold mutations in the fruit fly, *Drosophila melanogaster*. Neither can the effect of chilling on mitoses and mutation be described as specific. Similar anomalies have often been produced by narcotics (Gerassimow 1901, Sabline 1903) or by supraoptimal temperatures (Sabline 1903, Shimotomai 1927; see further X. 6*i*; X. 13).

In so far as chemical changes produced by low temperatures are concerned, they have been already discussed above (Chapter V; see further IX. 8).

5. Time-factor in the phenomena of chilling

As in other temperature actions, in the phenomena of chilling too, the action of the time-factor must be considered along with that of temperature itself. We have discussed above (IV. 8) some cases in which a time-factor is apparent when the rate of biological processes is decreased by cold. In the present paragraph we shall therefore deal only with the analogous cases, in which this rate becomes equal to zero in consequence of chilling, or in which the chilling produces morphological changes.

Askenasy (1890) noted that the growth of roots in *Zea mais* at temperatures in the vicinity of 0° ceases more or less completely only after one to five hours. According to Klemm (1895), protoplasmic streaming is not arrested at once when the cells are cooled to —2°, but only gradually. Ewart (1896) observed that the O_2 production of certain tropical plants, cooled to 5°, fell to zero in one hour, but on cooling to 1°, O_2 production ceased in a quarter or half an hour. Certain flagellates (*Monas*), when kept at 4°, lose their motility in about one hour (Greeley 1902*b*). A frog muscle, kept at 0°, completely loses its excitability after 8—14 days, as was demonstrated by Foster and Moyle (1921).

The relation between the degree of cooling and the exposure necessary to bring to a stop the pulsations of explants from an embryonic chick heart, is shown by the following table (Bucciante 1927*b*):

TABLE LXV

Temperature	Time
$<12^0$	almost immediate effect
12—13°	6½ days
18°	10 days
21°	12 days
25—26°	12—29 days

Bělehrádek (1928*a*) has described a reversible arrest of the heart beat in *Daphnia pulex* on cooling the animal to 4°. The rhythm gradually slowed for about two hours before coming to a complete stop, though the temperature must have reached equilibrium in less than 10 minutes. In another

Cladoceran, *Moina macrocopa*, L. A. Brown (1929*a*) has noted that a complete loss of motility occurred at 10° only after one hour.

As may be concluded from these data, chill-coma does not develop unless a definite time elapses at a given temperature. Similarly death by chilling does not take place unless the organism has been chilled for a certain, usually a long, time (see above, IX. 3), which, too, depends on the temperature applied. Thus it was found by Back (1916) that the eggs and the larvae of the fruit fly *Ceratitis capitata* die when chilled at various temperatures for the following periods:

TABLE LXVI

Temperature	Lethal exposure
0—1°	2 weeks
1—4°	3 weeks
4—7°	7 weeks

Fig. 51, constructed from Robinson's (1926) data, is of a special interest in this connection, because it permits a comparison of temperatures above and below zero.

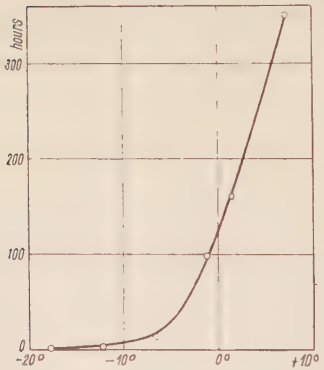

Fig. 51. Life duration (ordinate) of the rice weevil, *Sitophilus oryza*, at low temperatures (abscissa) below and above 0° C. Robinson 1926.

Likewise the morphological changes, which are connected with chilling, obviously develop with a considerable time-factor. In Greeley's experiments on protozoa (1902*a*, *b*, 1903*b*) the changes described above (IX. 4) took place only after one to several hours. Likewise Ishikawa (1913) reported that the change in shape due to chilling of an *Amoeba* took place slowly in about one hour. The pseudopodia first became shorter and brighter, then nodosities appeared on them, and finally no more pseudopodia were formed, the body assuming a spherical shape. Analogous experiments by Arens (1929*b*) on the spores of *Plasmopara viticola* showed that six hours are necessary for

the completion of these changes. Baldi (1930) has recently studied the effect of a low temperature upon the structure of a nerve, and has seen that the penetration of myelin in the interior of the nerve began after three hours of exposure and that it increased with longer exposure.

It is obvious that in all the instances quoted the time-factor greatly exceeded the time necessary for the temperature equilibrium.

The time-factor intervenes also in the process of recovery from chilling. But in this respect two cases must be distinguished. In the one case recovery takes place much more quickly than did the previous chilling, whereas in the other, the recovery, too, is slow. As for the first group of cases, Maurel and Lagriffe (1899) have noted that in fishes the recovery from a chill-coma is rapid. According to Ishikawa (1913), a chilled Amoeba recovers very quickly, when transported to a higher temperature, yet in certain individuals the time required for a complete recovery may reach even one hour. Stevens (1917*a*) has observed that the mycelium of *Endothia parasitica*, which had been previously cooled until its growth had ceased, started growth immediately on transfer to room-temperature. Bělehrádek (1928*a*) has noted in the heart of *Daphnia* a very rapid return to normal activity even after the heart had ceased to beat and where chilling had taken place slowly. The arrest of the heart-beat occurred at 4° after 110 minutes, while a complete recovery was attained in 4—10 minutes. In another Cladoceran, *Moina macrocopa*, L. A. Brown (1929*a*) observed chill-coma in about 4 minutes, whereas the recovery took place in 3 seconds. Yet in another species, *Pseudosida bidentata*, the same author saw that the recovery was almost as slow as the development of chill-coma. A slow return to normal activity has been similarly observed by Němec (1899) in the growth of roots in *Vicia faba*, by Joseph and Prowazek (1902) in the frequency of the pulsating vacuole of *Paramaecium caudatum*, by Bucciante (1927*b*) in pulsations of the embryonic heart cells of the chick. Figure 52 is reconstructed from one of the experiments of Joseph and Prowazek.

It is important to note that the rapidity with which recovery takes place often depends on the intensity of the previous chill-coma. The lower the temperature, or the longer the coma lasted, the slower the recovery. Thus Askenasy (1890) observed that the

growth of roots in *Zea mais* resumed its original velocity after a period whose length varied directly with the degree of chilling. Likewise Ewart (1896) noted that specimens of *Helodea*, which

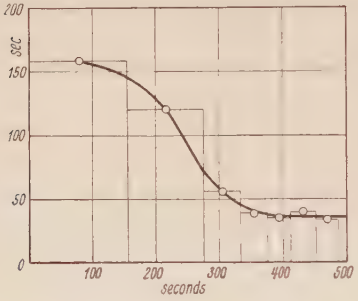

Fig. 52. Time factor in the return of the rhythm of pulsating vacuole to the normal frequency after chilling. Abscissa: time elapsed since the transfer to 8—9°. Ordinate: duration of intervals between two contractions. Constructed from an observation of Joseph and Prowazek.

had been chilled for a longer time, required a longer period for recovery, than specimens in which the chilling had been of short duration:

TABLE LXVII

Exposure to 0°	O_2 production reappeared, on transfer to 20°, after	Protoplasmic streaming reappeared, on transfer to 20°, after
6 hours	10—15 minutes	5 minutes
1 day	3 hours	5 minutes
2 days	3 hours	3 hours
5 days	5—24 hours	3 hours

Similarly Götze (1926) has observed that, for a complete recovery, chilled honey-bees must be heated to a temperature which increases with the duration of the previous coma. Likewise in young specimens of *Alligator mississipiensis* the recovery requires 5—120 minutes according to the duration of the chill-coma, as reported by Weigmann (1929).

Relatively little is known of the part played by the velocity of cooling. Kaestner (1895) thinks that the chick embryo can endure a slow chilling better than a rapid one.

In spite of considerable data now available on the time-factor in the phenomena of chilling, present knowledge does not yet allow of an attempt at a mathematical analysis of the relation between the degree of cooling and the time of exposure necessary to produce a given change.

6. Internal factors in the effects of chilling

(*a*) *Age.*

The most important internal factor involved in the chilling is age. Yet there is no uniform rule expressing this relationship. PICTET (1893*b*) saw that older ant pupae were less resistant to cold than young ones, and that they died at 5° within a few hours. In plants, it has been found by EWART (1896) and by SELLSCHOP and SALMON (1928) that the older parts are more resistant, while NĚMEC (1901) and MÖBIUS (1907) have seen just the opposite. According to MORGAN (1902), the embryos of *Rana palustris* at the stage of 1—2 blastomeres soon die in the ice box, while blastulae withstand the same degree of cold better. DARESTE (1891) thought that the chick embryo becomes more resistant to cold as the moment of hatching approaches, obviously in connection with the development of the regulation of body temperature. But KAESTNER (1895) observed that very young chick embryos can resist a lowering of the temperature to 21° for a period which varied indirectly with the age of the embryo. Fig. 53, which is drawn from KAESTNER's data, clearly shows that the change is gradual.[1]) PAYNE (1930) has recently reported that in the egg of *Arbacia* cleavage takes place down to 12°, but cannot proceed further, at this temperature, as soon as the gastrula stage is reached. At 4° the cleavage is arrested even at the blastula stage. Thus the older stages are more sensitive to cold. As to the development of insects, BLUNCK (1924) states that the biological zero increases with advancing age, whereas D. LUDWIG (1928*a*) estimates that the cold hardiness of the Japanese beetle (*Popillia japonica*) is greatest in the full

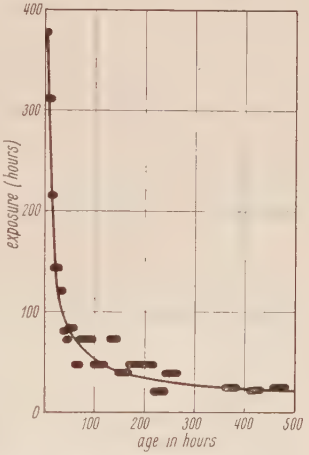

Fig. 53. Maximum exposure to 21° still supported by chick embryos decreases with advancing age. Constructed from data of KAESTNER.

1) This conclusion has been quite recently confirmed by KAUFMAN (1934).

grown first instar larva. The same author (1930) is inclined to believe, in common with Bodine (1925), that there are special critical periods in the development of insects, during which a lowering of the temperature is more injurious. Similar periods of increased sensitiveness to cold have been assumed by Stockard (1921) in the developing embryo of *Fundulus heteroclitus*. According to Bělehrádek (1928*a*), the heart of adult specimens of *Daphnia pulex* comes to a complete standstill at 4°, while that of younger specimens responds to the same conditions by a mere retardation of the rhythm. As to the mammals, it is now generally admitted that the young individuals can endure a much more considerable decrease in body temperature, than the adult organisms (Horwath 1876, Bunge 1905, Sinitzin 1923).

(*b*) *Adaptation.*

Organisms which are adapted to higher temperatures are naturally chilled more easily than those which live at low temperatures. Some examples of this may be found in Table LXIV (p. 139). It appears that the biological zero may be similarly shifted by an adaptation during the individual existence of certain organisms. Thus Bodenheimer and Klein (1930) find that the locomotion of the ant *Messor semirufus* ceases at the following temperatures at different periods of the year (see also fig. 28, p. 43):

TABLE LXVIII

Month	Minimum shade-temperature (Palestine)	Biological zero of creeping
I.—III.	9—10°	9.2°
IV.	13°	3.5°
V.—VI.	16—18°	11.1°
VII.—VIII.	21°	18.5°
IX.	19—20°	21.1°
X.—XII.	11—17°	8.4°

According to Hörstadius (1925), the development of the embryo of *Paracentrotus lividus* at the Naples Station is arrested by cold only at 8° in winter, but at 16° in summer. Similar modifications in the resistance to chilling by adaptation in plants have been studied by Wartenberg (1929).

Likewise during the hibernation of mammals the biological zero sinks considerably. The heart beat, which in normal active condition comes to a standstill at about 15°—17°, still continues when the hibernating animal is cooled to temperatures close to 0° (Tait 1922).

The true mechanism of these adaptive changes is not known. Tait (1922) has suggested that the essential factor here is the solidification point of the protoplasmic lipoids. We have actually seen (V. 3) that the solidification point of fats and phosphatids depends on the temperature at which they have been formed. This strongly supports the idea of Tait, which, moreover, is in agreement with the views put forward by Rywosch, Heilbrunn and Bělehrádek as to the rôle played by protoplasmic fats and phosphatids in the response of living systems to higher temperatures. Hörstadius (1925) assumes, in more general terms, that a change in the colloidal properties of the protoplasm takes place during the process of adaptation. These views will be discussed in more detail below (X. 17). On the other hand, Pantanelli (1919) suggested that plants rich in sugar are the most resistant to the injury produced by chilling.

(*c*) *Differences according to functions and regions of the organs.*

It has long been known that various functions of one and the same organism often have different biological zeros. Sachs (1864*b*) already had noticed that the production of chlorophyll in certain plants is arrested by cold at a different temperature from that which arrests germination. Semper (1880) has pointed out that in the pond snail (*Limnaea stagnalis*) growth ceases below 12°, while reproduction still continues. It is generally admitted that the growth of a plant is more easily checked by cold, than either respiration or photosynthesis are (compare Pfeffer 1904). For the rest, the reader is referred to Table LXIV (p. 139).

It is perhaps a more interesting topic, though one not often discussed, that various parts of one and the same organ are sometimes arrested in their functioning at unlike temperatures. Thus according to Unger (1912) the beat of the heart ventricle of a frog ceases at 10°, while that of the auricle only at 8°. The best studied example however is that of the nervous system. Winternitz (1905) was the first to note that when the body

temperature of a rabbit is artificially lowered, the highest nervous functions are put out of action first, while the spinal reflex action and with it the respiratory movements, disappear last of all. When a cat, whose temperature has been lowered to 16°, is warmed, the nervous functions reappear in the reverse order, as was demonstrated by Simpson and Herring (1905). The same phenomenon has been studied in much detail also by Britton (1922) in the cat and by G. E. Johnson (1929) in the squirrel. According to Artom (1927, 1928), the sympathetic ganglia of the cat are inactivated at 15°, while spinal ganglia only at temperatures below 7°. As regards poikilothermic animals, it is of interest to note that according to Weigmann (1929) recovery in chilled alligators proceeds from the distal parts of the spinal cord to the brain. In many fishes living in moderate climates, the sense of position is checked by cold sooner than motility, as stated by Maurel and Lagriffe (1899). Similarly Koike (1910) reports that in the electric cat-fish (*Malapterurus electricus*), the function of reflex centers is checked at 12°, while the breathing center ceases to function at about 10°. In a leech, *Hemiclepsis marginata*, Herter (1928) found that the posterior half of the body is the first to lose its motility when the animal is cooled to 0°. Götze (1926) observed that in the honey-bee the wing movements ceased at 15°, creeping ceased at 13°, the last isolated movements of the body at 8°—9°, the movements of antennae at 6°. Nobody has hitherto tried to study whether such differences in the chilling of various regions of the nervous system are associated with some differences in the cytological, chemical or colloidal properties which may exist between these different parts. Neither is it clear whether or not these differences are connected with the metabolic gradients discovered by Child.

(*d*) *Injury.*

As far as is possible to conclude from the scanty information now available, it seems that injured organs are inactivated at a higher temperature than those in the normal state. This has been demonstrated by Carlson (1906) for the heart ganglion of *Limulus* and by Bottazzi (1920) for the diaphragm of the dog.

7. Effects of chilling combined with external agents

There is sufficient evidence that the phenomena of chilling are sometimes modified by the action of certain substances.

The temperature minimum of growth in certain thermophilic bacteria is considerably lower in the absence of oxygen, as stated by RABINOWITSCH (1895). JOSING (1901) found that addition of ether causes a considerable delay in the cessation of protoplasmic streaming in cooled cells of *Vallisneria*. His results (fig. 54) show that the 1% ether solution is the most efficient dose. Likewise salts may exert a similar or a contrary effect. From the experiments of E. G. MARTIN (1904) on the heart beat of the terrapin it appears that the biological zero increases with the amount of KCl in the perfusion liquid. SELLSCHOP and SALMON (1928), on the other hand, have found that in plants the addition of potassium increases the resistance to chilling, while sodium and calcium decrease it. SCHAFFNIT and WILHELM (1933) recently have confirmed the rôle of potassium in the chilling of plant tissues and have observed furthermore a decreased resistance in excess of nitrogen and phosphorus. According to PAYNE (1930), the cleaving eggs of *Cumingia* become more resistant to chilling when the amount of $CaCl_2$ in the seawater is slightly raised to a concentration which at normal temperatures produces cytolysis. Is is obvious that the problem, regarded in another way, is, how does temperature modify the toxic effects of the substances examined. A substance, which is inoffensive at higher temperatures, may become toxic on cooling and thus brings activity to a standstill. We have already analyzed this point above (V. 9). F. WEBER (1932*b*) and MODER (1932) have recently described an injurious effect of urea on plant cells cooled to 1°—3°.

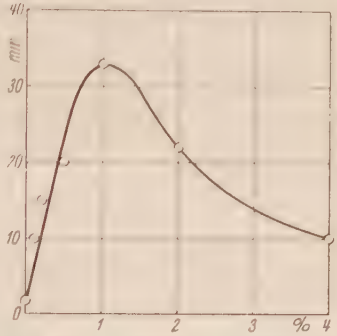

Fig. 54. Action of ether of varied concentration (abscissa) upon the time (ordinate) which is necessary to arrest protoplasmic streaming in *Vallisneria* on a sudden transfer to 1°. JOSING.

The relative amount of water contained in protoplasm, too, seems to modify the position of the biological zero. The most extensive work in this respect is that of SHELFORD (1927) on the development of the codling moth. As is shown by fig. 55, constructed from SHELFORD's data, the biological zero is at its

minimum within a humidity zone lying between 70 % and 93 %. Outside this zone, the biological zero rises, whether humidity be increased or decreased.

As to other external influences which may combine with chilling, Poynter and Moritz (1923) have observed that embryos

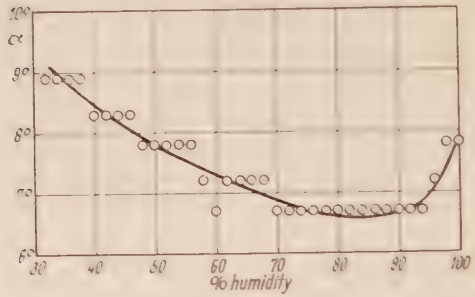

Fig. 55. Threshold temperature (α) for the development of the codling moth as a function of atmospheric humidity. Shelford.

of the pond snail (*Limnaea stagnalis*), previously irradiated by ultraviolet rays, died in two days at 7°, while at 24° they lived up to the fifth day. It may be that this is to be regarded as the combined effect of chilling and injury, already discussed above (IX. 6*d*).

8. Hypothetical causes of chilling

The earliest students of the phenomena described in this chapter thought that injury and death by cold without formation of ice is due to a disproportion between the velocities of several vital functions. Thus Sachs (1860*b*) suggested that the loss of turgescence in plants might be caused by a cessation of water intake by roots at a temperature which still permits transpiration of leaves to take place. Molisch (1896) however found that death by chilling occurs in plants even when the transpiration is eliminated by an atmosphere saturated with water vapour. Davenport (1897–1899) explained the temporary rigor by cold in animals as a combined effect of the stimulation by cold of certain vital activities, which cannot be satisfied because of lowered metabolism.

The overwhelming majority of modern workers however are unanimous in explaining chill-coma and death by chilling as a consequence of accumulation of toxic products, which at normal temperatures are burnt or eliminated. Nor is this idea quite

new. As a matter of fact, HORWATH (1876) supposed that mammals die when their body temperature falls below a definite limit, because a toxic substance is produced which paralyses certain important physiological functions. In the special case of mammals, no such substance has been actually found, but there is now much evidence at hand that the chemical composition of living matter and of its products is often changed when temperature varies. Numerous examples thereof have been summarised in Chapter V. Besides, certain modifications in the chemical composition of living systems have been revealed in connection with the special studies of the effects of chilling. MOLISCH (1896, 1897), PFEFFER (1904), J. LOEB (1906), LIDFORSS (1907), H. W. FISCHER (1911), PÜTTER (1911), CZAPEK (1913), HÖBER (1926), R. NELSON (1926), SELLSCHOP and SALMON (1928), LJUBIMENKO and HUBBENET (1930) and others merely admitted in more general terms that changes in the cellular metabolism, in the chemical composition of the protoplasm and of its products, in the solubility and in the adsorption of various substances, in the chemical equilibria inside the cell, in the relative velocity of catenary biochemical processes, etc., are responsible for the cessation of vital activities by cold and for death by chilling. PANTANELLI (1918, 1919) on the other hand undertook chemical analyses to demonstrate that in plants cold actually produces important biochemical changes. The main alterations described by this author are the accumulation of organic acids in the cell sap, formation of nitrogen by-products as a consequence of an increased decomposition of protoplasmic proteins, exosmosis of water and thus an increase in concentration of the cell-sap, and an increase in its permeability. More recent researches confirmed the loss of water and the increase in the crystalloid content as a consequence of chilling in plant tissues (MUDRA 1932, SCHAFFNIT and WILHELM 1933). It seems therefore that the changes produced by chilling in cells are complex and that none of the alterations can be said to be the ultimate cause of the phenomena of chilling. This seems to apply in the first place to the change in the water content which was made responsible for these phenomena by GREELEY (1902*a*, *c*), a pupil of J. LOEB, obviously with little justification.

On the other hand, FOSTER and MOYLE (1921), who examined the properties of a chilled frog muscle, did not detect any chemical

differences between the normal and chilled tissue, and they suggested, therefore, that the mechanism is rather more physical, involving a change in permeability or in the power to absorb ions. Tait (1922) thought that the melting point of protoplasmic lipoids played an essential part in the determination of resistance to chilling. It may be that the differences in resistance to chilling according to age can be explained on a similar basis, as it is known that the iodine number of fats and thus their melting point actually varies with age (Eaves 1910, Ssadikow and Golowtschinskaja 1928). Likewise Payne (1930) attributes the injury by chilling to changes in the protoplasmic fatty substances.

Of late much importance has been given to changes in protoplasmic viscosity, as being the possible cause of the phenomena of chilling. Such a possibility has been admitted by F. and G. Weber (1916), but especially by Heilbrunn (1924*a*), who had noticed a considerable increase in the viscosity of cytoplasm of the egg of *Cumingia* at low temperatures, and laid stress upon such "coagulative" change as being the probable cause of chilling. Similarly Artom (1928) suggests that the cessation of conduction in the nervous ganglia on cooling is due to some colloidal change in the synapses. Bělehrádek (1928*a*, *c*, 1931*a*—32*b*) has adopted the hypothesis that the cause of the cessation of certain vital phenomena due to cold is in general an increase of the protoplasmic viscosity or of that of certain protoplasmic phases. Such an increase would considerably hinder free movements of reacting molecules with the result that the biochemical reactions in the cell would be brought to a standstill. In support of his idea, he quotes the considerable time-factor which often accompanies the development of chilling (see IX. 5). Further he states that there is in general a close parallelism between the biological zero of a given activity and the numerical value of the corresponding temperature coefficient. It is obvious that, if this hypothesis is true, the biological zero will be the higher, the more viscous the protoplasm is at a given temperature. On the other hand, many facts can be cited in favor of the view that the temperature coefficients of biological processes are a function of the viscosity of the respective protoplasmic phases, as has been pointed out above (see IV. 9). The biological zero and the temperature coefficient are actually both modified by many factors,

internal and external, as we have already seen (Chapter III. — IV. 9; IX. 6; IX. 7), but the data now available are not yet full enough to justify the notion of complete parallelism. Still such parallelism seems indisputable at least in some cases, *i. e.* the effect of adaptation to temperature and the part played by the water content of the living system. The biological zero obviously increases with the temperature to which a given organism is adapted, and as a rule the temperature coefficient of vital processes varies accordingly (see III. 15). Similarly both the biological zero and the temperature coefficient increase when the amount of water in the protoplasm sinks, as is clear from the data of SHELFORD (1927) on the rate of development of the codling moth (compare figs. 15, p. 47, and 55, p. 283). It is important to note that when the atmospheric humidity exceeds a definite limit, there is again an increase of both the biological zero and the temperature coefficient. It is noteworthy that the supposed rôle of the protoplasmic fats and lipoids, mentioned above, agrees with the viscosity theory of chilling, because the viscosity of protoplasm must be expected to vary with the melting point of the fatty constituents (see further X. 17).

A similar comparison between the lower temperature limit and the temperature coefficient has been made, independently of BĚLEHRÁDEK, by ZAVADOVSKIJ and SIDOROV (1927, 1928) in the case of the embryonic development of certain lower animals, with the same result, yet they give no explanation for the phenomenon. It may be added that in general those biological processes which yield a high temperature coefficient, as for instance embryonic development, possess at the same time a high biological zero, and that conversely the processes with a low temperature coefficient continue down to very low temperatures, *e. g.*, conduction in nerves.

In this connection it is important to know whether the permeability of the cellular surface, too, is decreased to zero at low temperatures. It has actually been found by F. C. STEWARD (1932) that the penetration of bromine salts into the potato tuber is arrested at 3^0 to 5^0. The author admits that an increased protoplasmic viscosity might be the cause. Yet it seems that when the simple cessation of vital activity is followed by an injury, there is again an increase in permeability, as found by PANTANELLI (1919).

If the viscosity theory of chill-coma proves true, the possibility still remains that the increase of viscosity and the decrease of permeability bring about disturbances in the biochemical state of the cell and that thus the actual cause of the phenomena of chilling in many cases is ultimately chemical. This is made still more probable by the fact that the position of chemical equilibrium is changed when the viscosity of the medium is varied (see Pissarjevski 1908).

CHAPTER X

INJURY BY HEAT AND HEAT RESISTANCE

1. Meaning of the terms temperature optimum and maximum

Although there is general agreement that life processes cease to remain "normal" as soon as a certain temperature is passed and that life is impossible at too high a temperature, there is much confusion as to the meaning of the temperature optimum and temperature maximum.

The meaning of temperature optimum originally was ecological, and it was used to denote the temperature at which a given organism can best exist. Later the term was used in the physiological sense to designate the temperature at which a given process takes place with the greatest velocity. In this sense the temperature optimum is identical with the "celerrimum" (Blaauw 1926). Yet in some cases the optimum on the contrary is identical with the "tardissimum", so *e. g.* in the longevity of cells. The term becomes still more vague in meaning because of the fact that certain processes, as for example photosynthesis, seem to possess several temperature optima (Lundegårdh 1927, Walther 1927, Yoshii 1928). It will be shown below (X. 4) that the temperature optimum decreases with time, the change being often considerable, so that when speaking of temperature optimum, one should mention the length of exposure concerned (Lehenbauer 1914). In regard to this, Kuijper (1910), Leitch (1916), Fawcett (1921) and others (compare also F. F. Blackmann 1905) have suggested taking for an optimum the highest temperature which acts without any such time-factor. It must be remembered, however, that a considerable time-factor is sometimes observed even at temperatures which are very distant from the optimum. F. F. Blackmann (1905) proposed defining the optimum

as the highest temperature at which there is no permanent injury, the action being completely reversible. Yet he himself is aware of the difficulties which such a definition would imply in actual practice. In the special case of developmental processes, there seem to be many more difficulties in defining the temperature optimum, especially as the rate must be considered along with the degree of normality (see JANISCH 1931). It is clear that to define the optimum as being the temperature which is preferred by an organism when left to choose among several temperatures (MARTINI 1916, HERTER 1924, 1925, BODENHEIMER 1929, NIESCHULZ 1933), involves a still further complication.

In the following pages the term "temperature optimum" is to be understood as being identical with the inflection point of the temperature-velocity curve. In this sense the term is nowadays most often used in biology.

The term "temperature maximum", too, was used originally in the ecological sense, and it became more and more indefinite when it was understood in a physiological sense. A cell may be killed instantaneously at 100^0, or only after a long exposure at 50^0. The time-factor which accompanies the action of high temperatures (see X. 4—X. 5), thus again makes an exact determination of the temperature maximum impossible, unless the time of exposure is known (compare SACHS 1864*a*). In regard to this, certain authors determined as "lethal" the temperature which is sufficient to kill a given organism in one hour, while others suggested a shorter exposure, say one minute. When the suspension of many cells, or multicellular organs and tissues, are dealt with, still another difficulty arises, namely that different cells as a rule show unequal sensitivity to heat. In such cases it is a matter of convention to choose the moment at which all, one half, or one third, of the cells are killed (AYERS and JOHNSON, 1924). Another complication arises from the fact that an exposure to heat first leads to heat rigor (X. 3), which may be either reversed or followed by death, according to circumstances which are not always sufficiently taken into account.

2. Maxima

In considering what has been said in the foregoing paragraph, it is questionable how much one is authorised to compare the numerous data now at hand concerning the temperature maxima

and optima of various vital activities. The highest degree of reliability naturally is to be ascribed to data as to permanent exposure, during the whole life or during the development, to a given temperature.

Such data, which are obviously of a rather ecological interest, have been reviewed chiefly by DAVENPORT (1897—1899), DAVENPORT and CASTLE (1896), PFEFFER (1901), TIGERSTEDT (1910—1914), PÜTTER (1927), etc. Yet it will not beout of place to quote here a few figures which are perhaps important from a more general point of view.

TABLE LXIX

Species	Permanent existence still possible, or quite no longer possible, at	Remark	Authority
Fusarium (moulds), various species . .	33—35°	growth of mycelium	TOGASHI 1931
Bacillus anthracis. .	44°	cultivation	PASTEUR (*cf.* LEFÈVRE 1911)
Bacillus thermoamylolyticus	65—68°	cultivation	COOLHAAS 1928
Thermophilic bacteria	60°—>70°	cultivation	MISCHUSTIN 1927
Bacteriophage, thermophilic	>70°		ADANT 1928
Phormidium laminosum	>73°	(Thermal alga)	R.B. HARVEY 1924
Phormidium (sp.) . .	89°	(Thermal alga)	R.B. HARVEY 1924
Dunaliella viridis . .	50°	(Volvocales)	BAAS-BACKING 1930
Ulothrix (sp.) . . .	17°	(Alga)	OLTMANNS 1923
Phaeocystis poucheti .	11.6°	(Alga)	OLTMANNS 1923
Nitschia putrida . .	30°	(Diatom)	OLTMANNS 1923
Fucus	30°		OLTMANNS 1923
Didymium nigripes .	18—20°	(Myxomycete)	SKUPIEŃSKI 1930
Paramaecium aurelia	31.5°	development	WOODRUFF and BAITSELL 1911
Herpetomonas donovani.	34°	cultivation	CHRISTOPHERS and *autt.* 1926

As regards the maxima of single vital functions, they are not comparable with the same degree of reliability. In Table LXX only certain results, in which the timefactor was given are quoted.

TABLE LXX

Species	Activity not possible beyond	Exposure	Authority
		(*a*) Protoplasmic streaming and ameboid movement	
Nitella flexilis . . .	>37.5°	>100 seconds	Romijn 1931
Amoeba (*vespertilio*?)	50°	<5 min.	Van Herwerden 1927
Leucocytes of rabbit	55°	5 min.	Ono 1929
Leucocytes of rabbit	50°	9—30 min.	Ono 1928
		(*b*) Ciliary and flagellar movement	
Paramaecium caudatum	34—37°	several hours	Michelson 1928
Paramaecium caudatum	33°	1—2 days	Michelson 1928
Arbacia pustulosa, sperm	43.8°	1.9 min.	Bělehrádek 1930*b*
Human spermatozoa	40.2°	4 hours	Stigler 1915
		(*c*) Cellular division	
Fibroblasts (chick embryo)	—50°	20 min.	Kemp and Juul 1931*c*
Osteoblasts (chick embryo)	- 52°	3½ min.	Pincus and Fischer 1931
Malignant tissue (rat)	44°	3 hours	Westermarck 1927
Normal skin (rat) . .	46°	3 hours	Westermarck 1927
		(*d*) Excitability	
Frog nerve	40—42°	2 min.	Alcock 1903
Frog nerve	50°	30—45 sec.	Hafemann 1908
Frog nerve	40°	15 min.	Mansfeld and Hecht 1926

Species	Activity not possible beyond	Exposure	Authority
		(*e*) Heart beat	
Tortoise	48.6°	24 sec.	Athanasiu and Carvallo 1897
Tortoise	51.0	19 sec.	Athanasiu and Carvallo 1897
		(*f*) Metabolic processes	
Ulva, photosynthesis	45°	2 min.	Wurmser and Jacquot 1923
		(*g*) Killing of various types of cells (compare also *a*—*c*)	
Bacterium paratyphi B	60°	5 min.	Oerskov 1925*b*
Bacillus tuberculosis	63°	45 min.	Oerskov 1925*c*
Helodea densa, epidermal cells	55°	1 min.	Collander 1924
Helodea densa, leaf cells	54.3°	3 min.	Bělehrádek and collab. 1935
Beta vulgaris, root cells	55°	4.3 min.	Collander 1924
Draparnaldia glomerata (alga)	55°	⅓ min.	Collander 1924
Tradescantia discolor, epidermis	55°	44 min.	Collander 1924
Paramaecium caudatum	44°	9 sec.	Port 1927*b*
Paramaecium, sp.	43°	25 sec.	Poyarkoff 1928
Strongylocentrotus purpuratus, unfertilized eggs	32°	1.5—2 min.	J. Loeb 1908*a*
fertilized eggs	32°	1.5 min.	J. Loeb 1908*a*

3. Heat coma

A vital activity which has been arrested by heating the organism, may recover if the exposure was not too long or the temperature too high. This reversible change, now called "heat coma", "heat rigor", "heat immobility", "*Wärmestarre*",

"*Wärmenarkose*", "*Wärmelähmung*", was first observed, so it appears, by A. P. DE CANDOLLE (1806) in the excitability of *Mimosa pudica*. The plant loses the ability of response when kept at 37° during 11 hours, but resumes it on transfer to a lower temperature. Later on, the same phenomenon, *i. e.* the loss of spontaneous motility or of excitability, was noted in animals (PICKFORD 1851, KÜHNE 1859, 1864, CLAUDE BERNARD 1878, DALLINGER 1887) and studied microscopically in lower animals and plants. NAEGELI (1860), SACHS (1864*a*) and KÜHNE (1864) were first to study the microscopical changes which possibly might the occur in the protoplasm before and during the heat coma, but their observations did not reveal anything which could be considered as the intimate and specific cause of the phenomenon. As regards the more recent investigations in the same field, they, too, failed to give conclusive results.

Different organs of higher organisms often show unlike sensitiveness when subjected to heat, and it seems that the central nervous system is again the least resistant. Heat coma in the metazoa is ascribed to the temporary suppression of the activity of nervous centers (compare WINTERSTEIN 1902, 1905, BONDY 1904, FRÖHLICH and KREIDL 1921, H. MEYER 1909, 1927, BREMER and TITECA 1930). In this respect the effect of heat is similar to the action of cold (see VIII. 7, *d*: IX. 6, *c*).

Under certain conditions, not yet sufficiently narrowly defined, the effect of high temperatures leads directly to death, without any noticeable reversible coma (MANSFELD and HECHT 1926 in the nervous system, BREMER and TITECA 1930 in amphibian nerve, WALLACE 1931 in *Mimosa*). It is to be concluded that in such cases the heat coma actually does exist, but that it is of such short duration as to escape investigation.

Heat-coma must be considered as the external aspect of reversibility from heat injury, a more general phenomenon whose other aspects will be analysed below (X, 12).

4. Time factor in the action of heat

The changes which take place in living systems on warming to a high temperature do not occur at once, but require a certain length of time. It is thus important to examine the development of such changes in time.

There is firstly an abundant store of evidence that the temperature optimum sinks when the duration of the experiment is sufficiently prolonged. This was shown first by CHUDIAKOW (1894) for the production of CO_2 by yeast, by BLACKMAN (1905) and MATTHAEI (1905) for the assimilation of CO_2 by green plants (see also JOST 1906), and later on was demonstrated in various vital phenomena by many other workers (BUGLIA 1911 in the contraction of the chick oesophagus: FAWCETT 1921 in the growth of moulds: LJUBIMENKO and HUBBENET 1930 in the formation of chlorophyll: etc.). The following figures are taken from BUGLIA's paper on the chick oesophagus contracting in RINGER's solution:

TABLE LXXI

Duration of the experiment	Optimum temperature for	
	amplitude of contractions	frequency of contractions
1 hour	22°	25°
3 hours	17°	24°
6 hours	14°	21°

The contractility evidently suffers in this case sooner than the rhythmicity.

Secondly there is a certain number of papers dealing with the decrease in velocity of various vital activities when the system is kept at a supraoptimal temperature for an increasing length of time. Fig. 56 is a graphical representation of the results gained by VAN AMSTEL and ITERSON (1911*a*) on the production of CO_2 by yeast. There is no depression of the activity at 45°, but from 46° onwards the depressing effect of heat increases regularly with the time. The change seems to

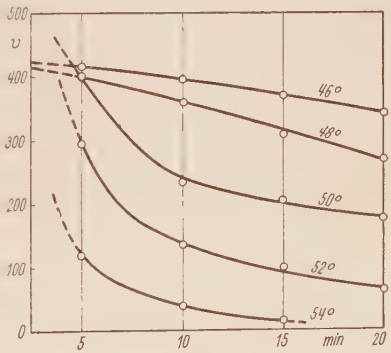

Fig. 56. Sinking of the activity of yeast cells at higher temperatures. Abscissa: time. Ordinate: velocity of fermentation. (VAN AMSTEL and ITERSON.)

follow an S-shaped curve whose form varies with the temperature. Yet the decrease in velocity in this and all other instances cannot be expressed mathematically by any simple formula, as pointed out by KANITZ (1915, p. 24). The depression of vital activity as a function of exposure to a given supraoptimal temperature has been studied further by RUTGERS (1911) in the "time of presentation" of the geotropic response in plants (compare also VAN AMSTEL and ITERSON 1911*b*), by WURMSER and JACQUOT (1923) in the rate of respiration in *Ulva*, by KLOPP (1924) in the bioelectric potentials of the frog skin, by KURBATOV and LEONOV (1930) in the respiration of seedlings of *Phaseolus aureus*, etc.

Another point which needs to be cleared in this connection concerns the rate at which a suspension of cells is killed when exposed to a supraoptimal temperature. ARRHENIUS (1903, 1915) and also some biologists after him argued that the destruction of blood-cells, sea-urchin eggs or bacteria by heat or any other agent follows a course which is identical with that of a simple chemical reaction, and they applied to the results thus gained the quantitative laws which are used in chemistry for molecular reactions of various orders. Yet GROS (1909) pointed out quite reasonably that a cell does not behave as a simple molecule, and that the resemblance of quantitative relations in this case is only superficial. Likewise KANITZ (1915, p. 98) refuted similar attempts and showed that one and the same type of quantitative expression may be obtained not only for monomolecular reactions and for the killing of cells, but for any process as well whose course depends on simple probability. Also VAN AMSTEL and ITERSON (1911*a*) expressed the view that the curve of survival percentage of yeast cells, acted upon by heat, is analogous to the curve of probability of GALTON. GROS (1909) attributed to the differences of age of the blood corpuscules the fact that haemolysis does not take place at once, but progressively, and MOORE (1917*b*) showed that age differences in the eggs of a starfish are responsible for the sigmoid curve representing the relation between time and percentage cytolysis. The law of probability is to be applied similarly to PORODKO's experiments on seeds (1926*c*). In reality the S-shaped curve which is often met with when the destruction of many cells or organisms by heat is studied, may easily be transformed into a curve of probability (fig. 57). This

complicated question, studied by still more complicated methods, is thus to be reduced to the simple statement that organisms of

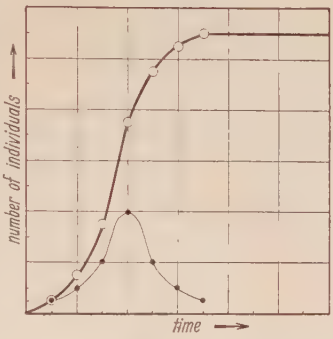

Fig. 57. Diagram to illustrate the relationship between the curve of frequency distribution and the sigmoid curve often met with in biological experiments on the rate of killing of organisms. Lower curve: number of individuals which are just able to withstand a definite exposure (abscissa) to a given injurious temperature. Upper curve: number of individuals found dead at the end of various time intervals. The upper curve is obtained from the lower one by progressively adding the respective numbers indicated for single time intervals.

a given kind possess unlike degree of heat resistance, obeying the laws of variability (see further X. 15*d*).

5. Temperature coefficient of heat action

The well-known fact that the velocity of heat injury depends on the degree of temperature applied (Sachs 1864*a*), may be represented by means of temperature coefficients. To this end several methods have been proposed:

(*a*) The simplest method is to determine the Q_{10} in the ordinary way (see II. 4). This method was adopted first by A. Meyer (1906), who determined the rate of killing of bacterial spores at high temperatures and calculated the ratio between the rates found at 80^0 and 100^0 respectively. He also compared the results of earlier workers and concluded that the duration of life decreases in geometrical progression, when the temperature is raised arithmetically. The idea was soon put on a more general basis by Jacques Loeb (1908*a*), Chick (1908, 1910), Moore (1910*b*, *c*), Goodspeed (1911), Lepeschkin (1912), Kanitz (1915), and others. The earlier work has been reviewed by Kanitz (1915); among the more recent publications should be mentioned first and foremost those by Groves (1917), Jacobs (1919), Bigelow (1921), Lepeschkin (1923*d*, 1924), Przibram (1923), Collander (1924), Klopp (1924) and de Visser Smits (1926).

In all the cases examined a high value of Q_{10} was found, as is now well-known fact. The lowest values were found in the killing of dry tardigrades ($Q_{10} = 2{,}8$) and of bacterial spores ($Q_{10} = 4$—12), the highest ones in the cytolysis of sea-urchin eggs by hot water (Loeb 1908*a*, $Q_{10} = 1.000$), in the killing of hydrozoa (Moore 1910*b*, $Q_{10} = 485$—3.900, compare Kanitz 1915) and of protozoa (Jacobs 1919, $Q_{10} = 58000$). — Yet in considering more closely the figures given by various authors it is evident that the Q_{10}, here too, is often very inconstant at various intervals of temperature (see Kanitz 1915, p. 107*seq.*: Jacobs 1919; Przibram 1923; Collander 1924; de Visser Smits 1926; Bělehrádek and Melichar 1930*a*).

(*b*) Arrhenius (1908, 1915) proposed his formula (see II. 7) to describe the effect of heat upon red blood corpuscules, and he has been followed lately by some biologists who studied other living systems: J. H. Smith (1923), L. A. Brown and Crozier (1927), L. A. Brown (1928), Krueger (1932). Bělehrádek (1928*b*) has pointed out that there is no advantage in the use of this rather complex formula in biology, because for narrow temperature intervals it may be reduced to the simple rule of van't Hoff (see II. 7).

(*c*). Porodko (1926*b*) suggested an empirical formula:

$$z = \frac{A}{t^m} \qquad \text{(XXXII)}$$

in which z is the time necessary to produce a given effect, *e. g.* death, t is temperature, A and m constants. The constant m has the meaning of a temperature coefficient. It will be noted that this formula is identical with that proposed by Bělehrádek (1926) for the change of velocity of vital processes with temperature (see II. 8), but this identity obviously is only accidental. The empirical formula of Porodko, originally designed for the killing of seeds at high temperatures and tested by the same author on some earlier data of other investigators (see fig. 58), has been tried since by Poyarkoff (1928) and by Bělehrádek and Melichar (1930*a*, *b*, 1931). The last mentioned authors have found in certain cases two different values of m, one for the temperatures not far from the optimum, the other, which is higher, for the highest temperatures examined. The same seems to hold good

also for several other organisms studied by other workers (fig. 67, p. 391; see also Collander 1924). The hypothetical conclusions, drawn by Bělehrádek and Melichar, will be referred to later (X. 16*f*).

(*d*). Janisch's temperature-time equation, already discussed above (II. 6) along with objections which have been raised against it, considers not only the biokinetic temperature scale, but also the supraoptimal temperatures. The chief obstacle in the practical use of this formula lies in the fact that the velocity of vital pro-

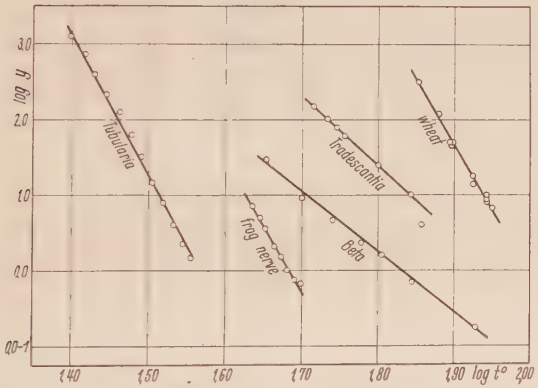

Fig. 58. Illustration of the validity of Porodko's formula. Abscissa = *log* of temperature. Ordinate = *log* of time necessary to produce death or a given degree of injury. Constructed from data of Moore 1910*b* (*Tubularia*), Hafemann 1908 (frog nerve), Lepeschkin 1912 (*Beta*, *Tradescantia*) and Groves 1917 (wheat grains containing 12% of water).

cesses at higher temperatures is not constant, but varies considerably with time of exposure, as has been shown in the preceding paragraph (X. 4).

It is perhaps superfluous to state expressly that none of these formulae can be regarded as rational and that all are only empirical equations. For this reason a general validity or a more general interpretation cannot be ascribed any of them.

It may be useful to quote here the chief temperature coefficients of heat action in tabular form (Table LXXII).

TABLE LXXII

(Temperature coefficients of the rate of injury by heat)

Species	Temperature coefficient			Temperature range	Authority
	Q_{10}	μ	m		
Spores:					
Bacillus anthracis	10.7[1])	—	—	90.4—105.3°	Ballner 1902
Various bacteria	5.6[1])	—	20.0[1])	100°—140°	Christen (*cf.* Matisse 1919)
Clostridium botulinum	9.5[1])	—	23.5[1])	100°—115°	Dickson and collab. 1925
Bacillus subtilis	4.4[1])	—	18.0[1])	110°—140°	A. Meyer 1906
Bacillus robur	6.0[1])	—	21.5[1])	110°—130°	A. Meyer 1906
Bacillus anthracis, dry	2.9[1])	—	15.8[1])	120°—135°	T. J. Murray 1931
Bacillus anthracis, moist	3.8[1])	—	12.0[1])	90°—105°	T. J. Murray 1931
Clostridium tetani, dry	2.0[1])	—	10.0[1])	95° 105°	T. J. Murray and Headlee 1931
Clostridium tetani, moist	7.1[1])	—	12.0[1])	125°—135°	T. J. Murray and Headlee 1931
Seeds:					
Wheat, 12% water	10.1	53.000[2])	17.6[1])—17.8[3])	71° 89°	Groves 1917
Wheat	54.3—84.5[3])	—	21.8—24.3[3])	45° 60°	Porodko 1926*a*, *b*
Nelumbo nucifera	6.3[1])	—	16.0[1])	70°—100°	Ohga 1927
Barley	11.0	41.500[2])	14.9[1])		
Bacteria:					
Bacterium coli commune	12	—	—	48.4°—52.7	Chick 1910*b*
Bacterium typhosum	136	—	—	49°—59°	Chick 1910*b*
Staphylococcus pyogenes aureus	29	—	—	48.4°—52.4°	Chick 1910*b*

Plant tissues					
Ceramium tenuissimum . .	37.6	—	—	28°—38°	Ayres 1916
Helodea densa, leaf	31	73.000[1])	15.0[1])	35° 55°	Collander 1924
Helodea canadensis, leaf . . .	7.6, 50.0	50.700 90.000	6.12, 21.2	20°- 65°	Bělehrádek and Melichar 1930*a*
Draparnaldia glomerata. . . .	43	63.600[2])	16.1[1])	35°- 34°	Collander 1924
Pisum sativum, seedlings . .	118	94.500[2])	18.5[1])	40°—50°(Q_{10}) 35°—55° (m)	Collander 1924
Tradescantia discolor	26	73.800[2])	18.8[1])	50°—65°(Q_{10}) 45°—65° (m)	Collander 1924
Tradescantia discolor	12.8		9.2[1])	52° 70°	Lepeschkin 1912
Brassica oleracea, leaf	31	94.500[2])	22.3[1])	45°—60°	Collander 1924
Beta vulgaris, root	71	94.500[2])	26.0[1])	45°—60°(Q_{10}) 50° 60° (m)	Collander 1924
Beta vulgaris, root	79.8	—	8.8[1])[3])	45°—85°	Lepeschkin 1912
Beta vulgaris, root	1.8—15.0[1])	—	4.0[1]) 15.0[1])	25°—60°	De Visser Smits 1926
Spirogyra	14—38		15.4[1])	38°—50°	Lepeschkin 1923*d*
Protozoa:					
Paramaecium caudatum . . .	<1.000[1])— 58.000	34.600[2]) 217.000	—	36°—43°	Jacobs 1919
Paramaecium caudatum . . .	4.550[1])	—	34.0[1])	38°—44°	Port 1927*b*
Paramaecium caudatum . . .	891[1])	—	24.8[1])	36°—43°	Poyarkoff 1928
Metazoa:					
Tardigrades (dry)	2,8[1])	—	7.4[1])	60°—90°	Richters 1908
Asterias, larvae	1.667[1])	160.000[2])	—	32°—40°	Jacobs 1919

1) Calculated by the author of this monograph.
2) Calculated by L. A. Brown and Crozier 1927.
3) Calculated by Porodko 1926*a*, *b*.

Species	Temperature coefficient			Temperature range	Authority
	Q_{10}	μ	m		
Strongylocentrotus purpuratus, eggs	1.000	93.600—131.400[2])	16.3[1])	20°—32°	J. Loeb 1908*a*
Strongylocentrotus purpuratus, eggs	50—500	72.800—101.900[2])	15.4[1])	34°—40°	Moore 1910*c*
Arbacia pustulosa, sperm . .	31[1])	—	13.0	33.3°—43.8°	Bělehrádek and Melichar 1931
Tubularia crocea	540	116.200[2])	19.5[1])	25°—36°	Moore 1910*b*
Daphnia magna	—	187.700	35.2[1])	35°—41°	L. A. Brown 1928
Fundulus, contraction of chromatophores.	8.3[1])	—	7.6[1])	32°—41°	D. C. Smith 1928
Frog nerve, sensorg.	34.6[1])	—	18.0[1])	43°—50°	Hafemann 1908
Frog nerve, motor	63.1[1])	—	22.2[1])	44°—50°	Hafemann 1908
Frog leucocytes	5.5—6.8	—	18.0[1])	38°—50°	Ono 1928
Frog leucocytes		—	4.0[1])	20°—38°	Ono 1928
Frog skin, electric potential .	10.0[1])		10.2[1])	38°—52°	Kloff 1924
Frog, striated muscle	69.2[1])	—	23.2[1])	35.5—48°	Buglia 1909
Drosophila melanogaster . . .	-	107.900[3])	—	31°—35°	Loeb and Northrop 1917
Rabbit leucocytes	28.8[1])	—	17.2[1])	38°—60°	Ono 1927
Rabbit erythrocytes (NaCl) .	500[1])	63.700[4])	28.2[1])	47.5—52°	Gros 1909
Osteoblasts from chick embryo	900[1])	—	17.0[1])	47°—52°	Pincus and Fischer 1931
Human carcinoma	412[1])	—	26.8[1])	42°—44°	Mendel 1928

1) Calculated by the author of this monograph.
2) Calculated by L. A. Brown and Crozier 1928.
3) Calculated by Porodko 1926*a*, *b*.
4) Arrhenius 1908.

Groves (1917) suggested that the Q_{10} is lower in plants than in animals, and he put an ecological interpretation on this assumed property of plants. Yet when the Q_{10} of various animals and plants are compared, Groves' statement does not hold good. On the contrary it appears from Table LXXII that organisms in a dry or semi-dry state, both animals and plants, yield low values of Q_{10}, while organisms under normal conditions give high figures. In general, it seems that the numerical value of the temperature coefficient of injury by heat is often associated with the degree of resistance to heat, as may be concluded for bacterial spores from the data of A. Meyer (1906; see fig. 59; compare X. 14*a*, 17).

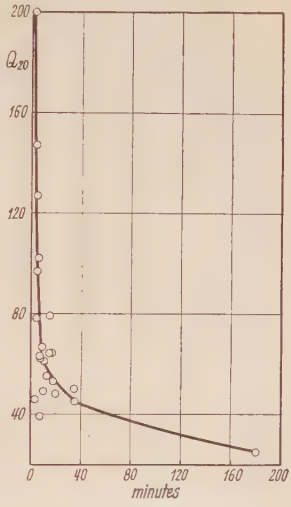

Fig. 59. To show the probable relationship between the degree of resistance of bacterial spores, expressed as the length of time necessary to produce death at 80° (abscissa), and the temperature coefficient (Q_{20}) of the rate of heat injury. Constructed from data of A. Meyer 1906.

Regardless of the formula applied, the temperature coefficients of the rate of injury by heat are always high, a conclusion arrived at first by J. Loeb (1908*a*) and by many others after him (for the earlier literature, see Kanitz 1915; compare further especially Arrhenius 1908, 1915; Chick 1910; Przibram 1923, Pütter 1927). When however the tissues are exposed to temperatures lying only slightly above the optimum, the temperature coefficient is lower.

6. Structural changes induced by heat

It was hoped, especially by the earlier investigators, that the mechanism of heat-injury might be revealed by the study of microscopical changes induced by heat. The extensive amount of work to which such attempts gave rise may be briefly summarized as follows:

(*a*) *Formation of granules*

High temperatures have often been shown to produce formation of new granules in the cytoplasm of various types of cells.

This was first demonstrated by M. Schultze (1863) in *Actinophrys eichhorni*. Yet Schultze thought that the granules were present even in the living cytoplasm and that they were only made more visible by high temperatures, from 43° onwards. At 35°—38° no new granules appeared, though a contraction of pseudopodia developed. H. de Vries (1871) described granules and turbidity in the protoplasm of plant cells killed by boiling. Analogous phenomena were observed later by Duclaux (1898—1899) in bacteria and by Klemm (1895) in the hair cells of various plants. On the contrary Khainsky (1911) stated that in *Paramaecium caudatum* there is an accumulation of crystalline excretory granules when the temperature of 24° is passed, but that at 30° the number of entoplasmic granules, which stain by neutral red or Bismarck brown, decreases. Lepeschkin (1910, 1923*d*, 1924) observed a formation of fine granules in the superficial protoplasmic layers of *Spirogyra* at 43°—47.6°, and Fauré-Fremiet (1924) in the egg of *Sabellaria* at 20°—33°.

(*b*) *Ultramicroscopical changes*

produced by heat were studied by Port (1927*a*) in *Paramaecium*. The surface layer, which normally is bright in dark-field illumination, becomes dark on heating. Runnström (1928*a*), working with the eggs of the sea-urchin, arrived at a similar conclusion. There is at the surface of the egg a white or yellow gleam, which disappears on heating to 41°—65°.

(*c*) *Vacuolisation*

Nägeli (1860) probably was the first to describe a vacuolisation of the protoplasm in *Nitella syncarpa* on heating to a temperature at which protoplasmic streaming ceases. The same phenomenon was observed by Strasburger (1878) in the zoospores of *Haematococcus* at 50°, and by Dallinger (1887) and Jahn (1933) in flagellates. Yet the vacuoles disappeared in the latter case as soon as the culture became adapted to the new temperature. Similar cytoplasmic vacuolisation, as a sequence of heating beyond the optimum, occurs in *Paramaecium* (Khainsky 1911), in many algae (O. Hartmann 1918*b*, 1919*c*), in the root-tip (Georgewitsch 1910), in the cells of onion scales (Wassermann 1921), in the sea-urchin egg (Ephrussi and Parat 1927), etc.

(*d*) *Protoplasmic contraction*

is another effect of heating which has been frequently described in the most diverse types of cells. The first observation of the phenomenon again is due to NÄGELI (1860), who found it in *Nitella*. Soon after him, M. SCHULTZE (1863) described permanent contractions and "varicosities" in the pseudopodia of *Actinophrys*. The phenomenon has since been observed chiefly in rhizopods by ISHIKAWA (1913) and VAN HERWERDEN (1927), in amebocytes of the starfish by FAURÉ-FREMIET (1929), and others. D. C. SMITH (1928, 1929, 1931) described the contraction in the melanophores of various vertebrates at high temperatures.

(*e*) *Changes of cellular size and shape*,

described in yeast cells by RICHARDS (1928), in ciliates by LÖHNER (1913, 1930) and in the eggs of the sea-urchin by KNAFFL-LENZ (1908), EPHRUSSI (1925*a*, 1927) and ACHARD (1927*a*), are to be explained either as a result of osmotic changes (LÖHNER, EPHRUSSI 1925*a*), or as a change in imbibition (EPHRUSSI 1927). These changes seem to be analogous to those which occur in the biokinetic temperature interval and which have already been discussed above (V. 2). On the other hand, the size or shape of certain cells varies on long exposure to slightly supra-optimal temperatures (MOLLIARD 1925 in *Aspergillus niger*; RICHARDS 1928 in yeast-cells; HAINES 1931 in moulds; etc.). Such changes perhaps may be compared with those which are discussed in Chapter VI.

(*f*) *Cytolysis*

is the final effect of heat injury and may often occur with great rapidity. It has been observed in the eggs of echinoderms by many investigators, and studied quantitatively on the same material by MOORE (1917*a*, *b*). In protozoa the incipient heat cytolysis is made manifest by the swelling of the body, which soon disintegrates (LÖHNER 1913, 1930; PORT 1927*a*, *b*; CHALKLEY 1930*a*, *b*). Yet the best-studied type of cytolysis of this kind is the well-known heat haemolysis (for the earlier work see KOEPPE 1903), which has been investigated quantitatively by KOEPPE (1903), GROS (1909), ARRHENIUS (1908, 1915), RYWOSCH (1911) and others. At 52°—55° the erythrocytes become spherical, exude colourless droplets and disintegrate into fragments which lose haemoglobin. The process is greatly hastened at higher

temperatures and at 68° the haemolysis is instantaneous (Koeppe 1903). When however still higher temperatures are applied, the interior of the cells coagulates at once and no haemolysis takes place.

Similar behaviour is observable in the ova of various marine animals. When the temperature applied does not greatly exceed the biokinetic limit, the egg is cytolysed. At very high temperatures, however, rapid coagulation occurs (Knaffl-Lenz 1908; Hörstadius 1923, 1925; Runnström 1924, 1928*b*).

(*g*) *Changes in lipoid constituents.*

Certain cytological changes produced by warming are apparently connected with the liberation of protoplasmic fats and lipoids. Chiefly at moderately high temperatures such phenomena are observed. Various cells, when heated, form at their surface round-shaped protrusions, containing a watery liquid and covered with a thin film, which probably is rich in lipoid substances. Such "hernial" protrusions, superficial vacuoles or myeline figures of various sizes are known to occur, under the action of heat, in the eggs of echinoderms (Knaffl-Lenz 1908, Runnström 1924, Hörstadius 1925), of *Ascaris* (Hogue 1910) and of certain annelids (Hörstadius 1923), in ciliates, in erythrocytes (Jolly 1926), etc.

Policard (1912) demonstrated that mitochondria in the hepatic and renal epithelium cells of the frog suddenly disappear on heating to 58°. The same change takes place in plant cells at 48°—50° (Policard and Mangenot 1922), yet the disappearance is not complete, and thin "shadows" remain, as was recently reported by Famin (1931). Likewise in the egg of *Sabellaria* there appear at higher temperatures (over 20°) granules, probably of a lipoid character, which disappear at 40° (Fauré-Fremiet 1924). Rounding up, fragmentation and other changes of shape, as well as loss of stainability, in mitochondria have been found to occur on the application of a temperature between 30° and 66° in cells of various organisms (Lewis and Lewis 1915; Famin 1931; Ephrussi 1933; compare reviews by Fauré-Fremiet 1925 and by Heilbrunn 1928). According to Heilbrunn (1924*b*) there is a very small "gray cap", composed of lipoid substances in normal centrifuged eggs of *Arbacia*, while in previously heated and then centrifuged eggs of the same species the "cap" is much larger,

or, on a more pronounced heat injury, it disappears completely. Likewise HOGUE (1910) found a marked difference in the stratification of the protoplasm in centrifuged *Ascaris*-eggs, according to whether they had been heated or not. Similar observations have been made by HÖRSTADIUS (1923) in the eggs of an annelid, *Pomatoceros triqueter*.

Lipoids seem to be altered in tissues of homoitherms during fever or sunstroke. FAHRAEUS (1926) reported that the fatty substances of the suprarenal tissue in rats first lose their anisotropy and then progressively disappear under the action of an artificial hyperthermia. That the blood during fever and sunstroke becomes rich in lipoid substances is a well-known fact.

The cellulose wall of plant cells, too, easily loses its phosphatids and sterols, when the temperature rises slightly over 30° (HANSTEEN CRANNER 1914, MAGISTRIS and SCHÄFER 1930).

(*h*) *Nuclear changes.*

The nucleus seems to be the most heat-sensitive of all cellular constituents (although EPHRUSSI 1933 maintains the opposite for the eggs of sea urchin). In the leucocytes of the frog, warmed to 37°, it goes into fragments and completely disappears, while the cytoplasm still moves, as was observed by RŮŽIČKA (1918). In *Spirogyra*, heated to 43°, the nucleus is the first to coagulate (LEPESCHKIN 1923*d*). CAPOBIANCO (1920, 1921) saw an extrusion of nuclei in the erythrocytes of the *Triton*, kept for several days at 34°. EPHRUSSI (1924*b*) described a moving of chromatin from the nucleus into the cytoplasm in the sea-urchin egg at 31°—32°. GREELEY (1903*a*) observed a fragmentation of the nucleus in the echinoderm eggs on heating them to 27°. Similarly DE LITARDIÈRE (1925) observed a dissolution of chromatin substance in the root-cells of the onion at 48°—50°. Some authors have observed a heat gelatinisation of the nucleus (ALVERDES 1922, VAN HERWERDEN 1927, NASSONOV 1932). According to VAN HERWERDEN (1927), new granules appear in the nucleus of *Amoeba* at about 50°. The same occurs in *Euglena* at 40° (JAHN 1933).

(*i*) *Changes in mitosis*

The changes just described concern the nucleus in the state of rest. Much attention was also paid to the action of heat upon the dividing nucleus. It is now a well established fact that the

dividing nucleus is more sensitive than the resting one (Němec 1909, 1910; Ephrussi 1924*b*, 1933; Buccıante 1926, 1927*a*; Kemp and Juul 1931*a*—*c*), as far as the cells of higher animals and plants are concerned. Lambert and Hanes (1913) established that the upper temperature limit for explants from chick embryo is more than 47°, but mitoses are arrested at 43°—44°. As regards protozoa, the only information available is that of Port (1927*a*), who found that conjugating *Paramaecia* are more resistant than non-conjugating ones.

The successive phases of the mitotic process are distinctly unlike in their response to heat. According to F. Wassermann (1921, 1931), the beginning and the end of the process are the most sensitive, while according to Ephrussi (1924*a*, 1933), Buccıante (1926, 1927*a*), and Kokott (1930), the more advanced phases are most easily arrested by heat. Kemp and Juul (1931*a-c*) have put forward the view that the critical moment is between the metaphase and the telophase. A satisfactory explanation of this behavior is not at hand, though F. Wassermann (1921) suggested that the phenomenon might be caused by differences in hydration, whereas the other investigators admit rather a colloidal cytoplasmic change, which presumably accompanies various stages of mitosis. Let us recall that various mitotic phases possess different temperature coefficients (III. 14).

When exposure to heat is not too long, the changes are reversible and the subsequent divisions are apparently normal (in the sea-urchin egg, Ephrussi 1924*a*, *b*; in explanted embryonic cells, M. R. Lewis 1933), but at other times asymmetric divisions of chromosomes may result, which may give rise to polyploidy. Atypical or asymmetric division of the chromatic substance has been observed by Schrammen (1902) and by Sabline (1903) in the meristem cells of *Vicia faba*, by Sakamura and Stow (1926) in pollen grains, by Ephrussi (1924*a*) in the sea-urchin egg, by Kemp and Juul (1931*a*—*c*) in the fibroblasts of the chick, etc. Polyploidy as the result of a semi-reversible heat-injury has been noted in various plants by Prillieux (1880), Koshuchov (1928), Cotner (1930*a*, *b*) and by others, in *Amoeba* by Daniel and Chalkley (1932), in embryonic tissue cells by M. R. Lewis (1933).

Anomalies of mitosis, induced by heat, might lead to hereditary modifications and to mutations. In fact many authors observed "permanent modifications" and true mutations as a result of the

exposure of organisms to a high temperature: in lower fungi (Haenicke 1916, Blochwitz 1923, Barnes 1928, Christensen 1929; earlier literature in Pfeffer 1901 and in Brierley 1920), in higher plants (Hiorth 1930, Hull and Grossman 1932, Navashin and Shkvarnikov 1933, etc.), in protozoa (Jollos 1913, Szabuniewicz 1929), in crustacea (Spooner 1932), in various insects (Standfuss 1902, Tower 1906, Goldschmidt 1929, Rokizky 1930, Efroimson 1932, Jollos 1932; compare also Ferry, Shapiro and Sidoroff 1930).

Effects of heat on chromosomes have been studied especially by Němec (1909—1910) and Milovidov (1932). Under the action of high temperatures, the chromosomes swell up and finally are dissolved.

7. Changes in protoplasmic viscosity

That supraoptimal temperatures increase the viscosity of the protoplasm, was first demonstrated by Russo (1910), who observed the gradual arrest of the Brownian movement in various vegetable and animal cells heated to 43°—50°. Heilbronn (1914) observed an increase in viscosity in certain plant cells, subjected to supraoptimal temperatures and examined by the method of falling starch grains. Later on (1917) the same author made a similar observation in plasmodia of slime-molds by means of his electro-magnetic method. Similar phenomena were later reported by other authors in various types of cells: by Heilbrunn (1924*a*,*b*) in the eggs of *Cumingia* and of *Arbacia*, working by the centrifuge method; by Fauré-Fremiet (1929) in the pseudopodia of amebocytes of the starfish, by microdissection; by Port (1927*a*) and by Van Herwerden (1927) in protozoa, by estimating the time of contraction of the pulsating vacuole; by Derry (1929) in *Spirogyra*, by the method of plasmolysis; by Tirelli (1930*b*) in *Arbacia* eggs by the centrifuge method; by Bělehrádek and Melichar (1930*a*) in *Helodea canadensis*, by studying the shape of plasmolysed protoplasts. Fig. 60 is a microphotograph reproduced from the last mentioned paper.

In spite of the many objections which may be raised against any of these methods, these results seem to demonstrate that the viscosity of protoplasm does increase on heating. The exception is the egg of *Sabellaria*, in which Fauré-Fremiet (1924) observed a liquefaction at temperatures higher than 28°. These tempera-

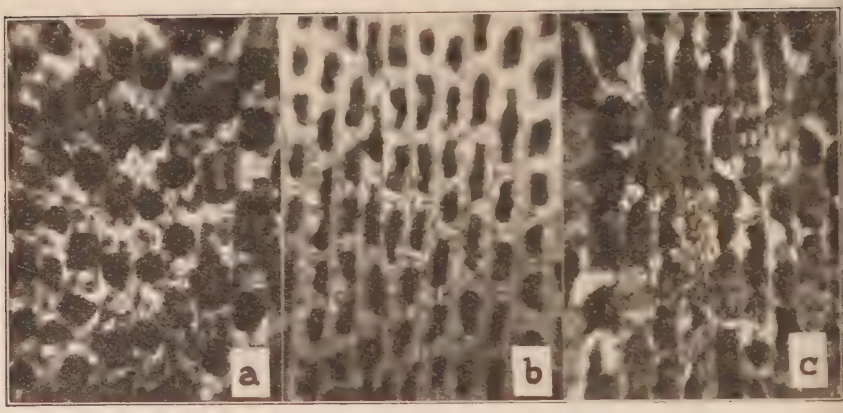

Fig. 60. Shape of plasmolysed and coloured (neutral red) cells of *Helodea canadensis*, showing various degrees of injury by heat.
a: normal, *b*: slightly injured, *c*: severely injured and dead (the latter do not stain). Bělehrádek and Melichar 1930*a*.

tures are decidedly supraoptimal for that species, as may be judged from the decreased rate of division and from the abnormality of the cleavage. Likewise Thornton (1932) saw a liquefaction in *Amoeba proteus* when warmed to 30°—38°.

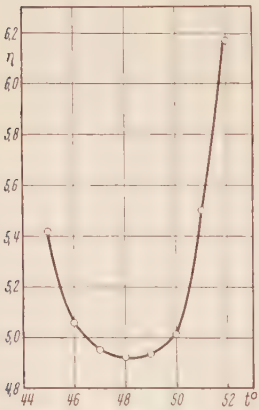

Fig. 61. Effect of temperature (abscissa) on the viscosity (ordinate) of the liquid expressed from eggs of the silkworm. Pigorini 1924.

An increase of protoplasmic viscosity by heat is in agreement with an analogous phenomenon not only in proteins, but also in liquids expressed *e. g.* from the ova of *Bombyx mori* (Pigorini 1924, 1931; Tirelli 1930*a*, *b*, 1931). The figure 61 is reproduced from Pigorini (1924).

It has been recently demonstrated by E. and H. Gellhorn (1928) that the temperature coefficient of the rate of penetration of vital stains through the frog skin at normal temperatures is considerably increased when the skin had been previously heated to 45° for 4 minutes. If it may be assumed that there is a relationship between temperature coefficients and the viscosity of protoplasm

(see IV. 9), this too would indicate an increase of protoplasmic viscosity as a consequence of heating the tissue.

8. Changes in permeability

It was known as early as 1855 to Nägeli that plant tissues, killed by hot water, lose their turgor and give off natural pigments (see Sachs 1864*a*). These phenomena now often serve as a test of vitality in experiments on the action of heat (Lepeschkin 1910, Collander 1924, de Visser Smits 1926, etc.). Stiles and Jörgensen (1917) observed that the intake of water by potato tuber tissue ceases and is replaced by exosmosis on heating to 40°.

When plant tissue is heated gradually, its electric conductivity increases, but only as long as the biokinetic temperature range is not surpassed. From 45°—50° onwards the increase becomes very rapid and the change in conductivity is now irreversible (V. H. Blackman and Paine 1918; Dixon and Clark 1928; Sen 1928).

In general there is an irreversible loss of selective permeability when the tissues are injured by heat. The surface of a cell killed by heat behaves almost as an ordinary lifeless membrane (Hewitt 1924). Yet E. and H. Gellhorn (1928) have observed that heating of a frog skin to 45° increases its permeability for chlorides and for acid dyes, but decreases the permeability for basic dyes.

9. Chemical changes

As temperature generally modifies the quantity and chemical composition of many metabolic products (see Chapter V), the question arises as to what extent heat injury is associated with such chemical changes.

de Vries (1884) observed the accumulation of acids in succulent plants exposed to a high temperature, and the same change certainly takes place in any plant when subjected to supranormal temperatures (see Pfeffer 1901; Iraklinow 1912; H. Evans 1932, etc.). Likewise in minced muscular tissue the production of lactic acid is greatly increased on heating (Tesauro 1924). Molliard (1925) observed a decrease of the ash content of *Sterigmatocystis* cultivated at supraoptimal temperatures. Parallel with this change was a modification in the chemical composition of the cell-wall, revealed by staining with iodine. Khainsky (1911) observed an accumulation of dark granules of

excretory products, often crystalline, in the protoplasm of *Paramaecium* at and above 24°. Scaffidi (1928*a*, *b*) and his collaborators d'Avanzo (1928, 1929), Gualdi (1928) demonstrated that chemical changes occur in various mammalian and frog tissues on the application of supraoptimal temperatures. In green plant cells formation of pheophytine from chlorophyll often occurs at high temperatures (Jahn 1933, Röben 1933).

Many other similar changes, chiefly in the water content, which accompany heat-injury, were studied in connection with the action of biokinetic temperatures. These have been discussed above (V. 2).

10. Changes in bioelectric potentials

As stated first by Engelmann (1872), the electromotive force of currents derived from the frog skin rapidly falls at 30°—35°, and disappears entirely at 40°. This was confirmed later by Bach and Oehler (1880) and is now known as a characteristic effect of heat upon living systems. Under appropriate conditions the study of bioelectric currents may become a suitable method for the investigation of heat-injury (Klopp 1924; see fig. 62).

11. Sequence of changes produced by heat

Löhner (1913) found that the symptoms of injury in ciliates develop in the following order, when the cells are gradually heated at the rate of 1° per minute:

TABLE LXXIII

Species	Maximum velocity of locomotion	Circus movements	Paralysis of cilia	Death
Colpidium colpoda	30°	35°	37°	38.3°
Leucophrys patula	31°	37°	39°	39.7°
Paramaecium caudatum	34°	42°	44.5°	45.0°

Lepeschkin (1923*d*, 1924) distinguishes four stages in what he calls "the heat coagulation of protoplasm", as may be observed in *Spirogyra* at 43°: (1) invisible change in dispersity, associated with an increased permeability; (2) visible coagulation of the superficial protoplasmic layers; (3) coagulation of the chloro-

plasts: (4) complete coagulation of the whole cell. Likewise in *Paramaecium* heat injury develops in four successive stages (Port 1927*a*): (1) increased permeability for water; (2) beginning of coagulative changes at the surface, circus movements; (3) immobility, swelling of the body, coagulation advancing from the poles inwards: (4) complete solidification of the whole body, followed by disorganisation, while the cilia are still in motion.

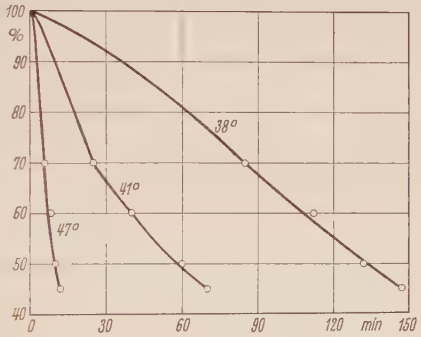

Fig. 62. The course of the process of heat injury of the frog skin, evaluated by means of the percentage lowering of the electric potential difference between inside and outside surface. Abscissa: time of exposure. Ordinate: percentage decrease of the potential reached in the time. Klopp 1924.

From other investigations, discussed in X. 6*h*—*i*, it appears that the nucleus is injured by heat before any other constituent of the cell.

It is noteworthy that cellular activity may be decreased or even lost before any structural changes can be detected. This was observed by Resvjakov (1923) for the excitability of the frog nerve and by Wurmser and Jacquot (1923) for photosynthesis in certain algae.

12. Reversibility of heat injury

We have seen above (X. 3) that the alterations produced by heat in various vital activities are often reversed on the transfer of the organism to a normal temperature. Likewise it was observed by numerous investigators, whose work has been discussed in the preceding paragraphs (X. 6—X. 11), that the structural, biochemical, colloidal and other changes produced by heat, are reversible, if they are not too advanced. The ability to recover is in some cases restricted to a short interval and thus cannot be noted experimentally, but in many other cases it is maintained for hours.

Apart from that, the recovery itself is rapid in certain instances, but may last for months in others. A. P. DE CANDOLLE (1806) reported that *Mimosa pudica*, made inexcitable by an exposure to 37° for 11 hours, recovered at 20° after one or two days. STRASSBURGER (1878) saw that zoospores of certain algae, immobilized by 50°, resumed their motility after 15 minutes at an ordinary temperature. In 1925, 1928, DICKSON and his collaborators observed what is probably a record period of dormancy (72 months) following injury by heat in the case of spores of *Clostridium botulinum*.

When a living system is heated repeatedly, three different cases may present themselves: (1) the effect is equal to the simple sum of all the single exposures, *i. e.* there is no recovery in the intervals between successive exposures, nor any adaptation; (2) the final effect is less than the single injuries, *i. e.* recovery takes place in the cold intervals; (3) the system increases its resistance with an increasing number of exposures or on application of increasing degrees of temperatures, *i. e.* the system becomes adapted. The third of these cases will be dealt with in a special paragraph (X. 17). The second case is of importance in this connection.

LEPESCHKIN (1923*d*) proved that filaments of *Spirogyra*, heated uninterruptedly, died after a shorter exposure than those in which exposure to the same high temperature was intermittent. JACOBS (1919) likewise demonstrated that *Paramaecia* die sooner when they are heated quickly and when the recovery process is thus eliminated, than when the heating is slow. That such recovery may be relatively rapid, is evident from KLOPP'S (1924) figures on the bioelectric currents of the skin of *Rana catesbiana*. The potential, which falls when the skin is exposed for 10 minutes to a temperature of 38°, is restored within 20 minutes at a normal temperature. When the fall in potential is more pronounced, the recovery is not complete, and when the injury is still more advanced, the potential difference of the skin is restored for only a short time, and is destroyed very soon afterwards.

JACOBS (1919) remarks that *Paramaecia* which were injured by heat to a definite stage may conserve their vitality for 24 hours at normal temperature, but finally die. On the contrary, EPHRUSSI (1924*a*—1925*b*) and ACHARD (1929) observed a normal or almost normal development of sea-urchin eggs which were previously

treated by heat, though they showed important colloidal changes. The same was demonstrated by TIRELLI (1930*a*) in the eggs of *Bombyx mori*.

According to *LEPESCHKIN (1923*d*) recovery is no longer possible in *Spirogyra* after the chloroplasts have begun to coagulate. On the other hand, the coagulation of the superficial protoplasmic layer may be reversed. PORT (1927*a*) observed that *Paramaecium* could be restored after injury by heat, if the stage of colloidal changes in the superficial protoplasm was not passed.

TIRELLI (1930*b*), who measured the protoplasmic viscosity in the eggs of *Arbacia* by the centrifuge method, observed, in agreement with the earlier results of HEILBRUNN (1924*b*), that the increase of viscosity was reversible in the beginning, *i. e.* at not too high a temperature and not too long an exposure, but when the eggs thus treated were subsequently fertilized, the reversibility was no longer possible.

13. Non-specific nature of heat-injury

Among the various modifications of living systems produceable by heat, there is none which might be said to be exclusively brought about by heat alone. In fact the same modifications can be induced by many other agents, physical and chemical. It was noticeable even to the earliest students of this problem that practically the same changes are produced in living bodies by extremely high as by extremely low temperatures (NAEGELI 1860, SACHS 1864*a*, HOFMEISTER 1867, KLEMM 1895). SACHS (1864*a*) further pointed out that certain cytological changes take place not only on the application of heat, but also on the application of electric current. Likewise atypical mitosis, polyploidy, etc. have been produced equally by high and by low temperatures (SABLINE 1903, EPHRUSSI 1924*b*, KOSHUCHOV 1928, BLEIER 1930, etc.) and certain chemicals, notably the narcotics, act under certain circumstances in the same way (SABLINE 1903, SHIMOTOMAI 1927). It would not be difficult to multiply evidence of non-specificity of heat effects by comparing other changes, which have been said to accompany the injury by heat, with similar changes which take place under the action of cold, mechanical injury (LEPESCHKIN 1924), various radiations, salts (PORT 1927*a*), diverse chemicals, differences in pH, high or low osmotic pressure, etc. (compare, in the case of the sea-urchin egg, EPHRUSSI 1924*a*;

as to the atypical coloration of butterflies: Greeley 1903*b*, Ziegler 1905, E. Fischer 1908, J. Loeb 1912, Prochnow 1927).

14. Effect of heat combined with other agents

Attemps at a hypothetical explanation of the effects of heat upon living systems made it necessary to study the action of high temperatures in combination with other agents. There is now at hand an extensive literature which it will be useful to review prior to the discussion of the theories of heat-injury (X. 16). From what has been said in the foregoing paragraph, it is to be expected that combination with any agent, physical or chemical, will in most cases increase or hasten injury by heat (Stigler 1915, Lepeschkin 1923*d*). This assumption is actually confirmed by numerous observations, as will be demonstrated below. Yet the effect may also be such that the agent in question increases the resistance of the system to heat, though this seldom actually happens.

(*a*) *Water content.*

Already Sachs (1864*a*) and H. de Vries (1871) quoted as a commonly known fact, etablished chiefly by Pasteur and others, that dry cysts, spores, seeds, dry tardigrades, yeast-cells, etc. resist heat better than moist ones. It is now current knowledge that such forms can be submitted to temperatures of 100 ° or even more without injury for a relatively long time. Sachs also pointed out that water plants are more easily injured by heat than dry land plants, a conclusion which however could not be accepted nowadays. It would be out of place to quote here all the extensive work hitherto published on the increase of resistance to heat with decreasing water content. (Compare especially: dry moss: Ewart 1896: — seeds: L. Just 1877, Groves 1917, Sigalas and Marneffe 1922: — pollen grains: Hiorth 1930: — dry yeast: Schumacher 1874, Schützenberger 1879: — bacterial spores: Sachs 1864*a*, Globig 1888, Lewith 1890, Bigelow and Esty 1920, Magoon 1926*b*, Dickson 1928, Dickson and collaborers, 1924: — bacteriophage: D'Hérelle 1926: — spores and cysts of protozoa: Dallinger 1880, Bodine 1923*a*: — moss fauna: Richters 1908, Rahm 1923: — eggs of certain crustacea: Mathias 1929. — Many data are collected in: Davenport 1897—1899, Davenport and Castle 1896, Pfeffer 1901—1904, Buchanan and Fulmer 1928—1930.)

Much time has been spent in studying the resistance of various insects under varied conditions of humidity (see the reviews by Buxton 1932, Imms 1932). It seems that at a definite atmospheric moisture-content the resistance of certain insects is at its highest and that it is lowered both on increasing and decreasing the humidity (Beattie 1928).

Bodine (1923*a*) studied more recently the susceptibility of protozoan cysts (*Colpoda cucullus*) to heat as compared with protozoans in the active state. Dry cysts withstood 100° for three days, moistened cysts died at $< 55°$, while active organisms were killed at 37°—45.6°. Similar results are those gained by Mathias (1929) with the eggs of phyllopods. Yet it is important to note that a decrease of water content by a hypertonic medium does not increase resistance to heat (see below, *g*).

The question as to whether the numerical value of the temperature coefficient of heat injury is influenced by the percentage of water present in the system, can best be answered by comparing the data contained in Table LXXII, p. 174. Generally speaking, dry organisms seem to yield lower temperature coefficients than organisms of high water-content. We arrive at the same conclusion in the case of Groves' (1917) experiments on wheat grains:

TABLE LXXIV

Amount of water in seeds	Q_{10} of the rate of killing
9%	9.2
12%	10.1
17.5%	16.5

(*b*) *Variations of pH.*

The resistance to heat of various lower organisms and isolated cells is greatly affected by pH-variations. Koeppe (1903) found that the addition of hydrochloric, sulfuric, formic, acetic and other acids lowered the temperature for instaneous haemolysis to 18°—37°, while at the normal pH it is necessary to heat the blood to as high a temperature as 68° to produce the same effect. The same is known for ammonia (Arrhenius 1908). According to Lepeschkin (1910), the filaments of *Spirogyra* are killed by heat at a temperature which varies with external pH thus:

TABLE LXXV

Medium	Chloroplasts coagulate at	External cytoplasm coagulate at
normal	47.3°	51.6°
0.1% Na_2CO_3	49.0°	55.4°
0.1% citric acid . . .	44.8°	46.0°

In this case resistance is decreased by the addition of acid, and increased slightly by the addition of an alkali. The decrease of resistance on addition of an acid was confirmed by KAHO (1924*a*) for cells of higher plants. Likewise MOLLIARD (1925) reported that the acid reaction depresses the temperature at which certain cytological changes occur in *Sterigmatocystis* as a consequence of heating. The same holds good for bacterial spores, as observed by BIGELOW and ESTY (1920), and for various bacteria in active form (B. COHEN 1922).

Thus it seems that at a definite pH, which is usually not too distant from the normal, the resistance is at its highest, and that it decreases both on lowering and on increasing the pH. The following table summarises the chief results:

TABLE LXXVI

Species	Maximum resistance at pH	Authority
Bacillus parathyph. B . . .	6—8	OERSKOV 1925*a*, *b*
Bacillus anthracis, spores .	8	F. J. MURRAY 1931
Clostridium tetani, spores .	7	F. J. MURRAY and HEADLEE 1931
Clostridium botulinum . . .	7	P. WEISS 1921
Bacillus anthracis	7	WITHWORTH 1924
Mammalian erythrocytes. .	7	JODLBAUER and HAFFNER 1920*a*, *b*
Paramaecium caudatum . .	7—8.2	CHALKLEY 1930*a*
Euglena gracilis	5.0	JAHN 1933

On the other hand, the resistance of *Dunaliella viridis*, a flagellate, seems not to be modified by pH between 6—11 (BAAS-BECKING 1930). Yet the value of pH at which the resistance to

heat-injury is highest, does not always agree with the optimum pH for the respective organism. For instance *Bacillus anthracis* shows maximum resistance at pH = 7.0, yet its development is optimal at pH = 7.8 (Withworth 1924). In *Euglena* the respective values are 5.0 and 6.6 (Jahn 1931, 1933).

Chalkley (1930*a*, *b*) demonstrated that *Paramaecium*, when subjected to heat, is destroyed by coagulation when the medium is acid, and by bursting when the medium is alkaline.

The temperature coefficient of heat injury, too, varies with the pH of the surrounding medium, as is clear from the figures of Orr (1918) on the killing of *Bacillus pseudotetanicus*:

TABLE LXXVII

pH	Q_{10}	
	80°—90°	90°—99°
3.0	5.0	9.3
4.3	6.3	8.9
6.3	5.0	8.4
6.6	6.8	8.3

Arrhenius (1908) quotes from the work of Madsen and collaborators that ammonia similarly modifies the value of μ for the velocity of heat haemolysis.

(*c*) *Action of salts*

M. Schultze (1863) suggested that the higher resistance to heat of fresh-water *Actinosphaerium* as compared with the sea-water species might be due to the action of salts, which would thus increase the sensitivity. de Vries (1871) showed that the resistance to heat in plants is decreased when the concentration of salts in the medium is raised. Yet more recent work tends to show that the action of salts is actually much more complicated. In this section the specific action of ions will be briefly summarised, while the osmotic effect will be discussed separately (X. 14, *g*).

Addition of salts to the medium regularly modifies the temperature optimum. The following table is taken from a paper by Greeley (1903*b*) on the combined action of temperature and salts upon the degree of swelling of frog muscle in isotonic media:

TABLE LXXVIII

Salt:	K_2SO_4 K_2CrO_4	Na_2SO_4	NaCl	KCl	NH_4Cl $MgCl_2$	LiCl
Maximum degree of swelling reached in 24 hrs. at t^0	19^0	21^0	22^0	24^0	23^0—25^0	27^0

The temperature optimum of the heart beat of the terrapin depends on the presence of potassium as follows (E. G. Martin 1904):

TABLE LXXIX

Percentage of KCl in perfusion liquid	0	0.03 (=normal)	0.08	0.11
optimum t^0	32^0	32^0	$>38^0$	28^0

J. J. Bouckaert, J. P. Bouckaert and Noyons (1922) observed that the beat of a frog's heart, stopped by the removal of calcium at an ordinary temperature, begins again spontaneously on cooling to -2^0. Very low doses of $CaCl_2$ produce the same effect, whereas with larger doses of the same salt the heart becomes more sensitive to higher temperatures than normally. There is thus an interrelation between the percentage of Ca and the temperature optimum, or, which is the same, between temperature and toxicity of Ca. Experiments with KCl gave a similar result. According to Fröhlich and Kreidl (1921), the heat coma of the heart of *Palaemon* develops at a lower temperature when the amount of magnesium is increased.

Richet, Bachrach and Cardot (1925) reported that in the bacteria of lactic fermentation the temperature optimum is elevated by the addition of KCl. Chalkley (1930*a*) saw in *Paramaecium* an increase in thermal resistance on the addition of Ca, and a decrease on the addition of K. Sodium chloride lowers the resistance of spores of *Clostridium botulinum* to heat (H. Weiss 1921). Calcium and iron seem slightly to increase the resistance of *Bacillus subtilis* (Williams 1928). Molliard (1925) demonstrated that potassium lowers the temperature at which

heat-injury occurs in *Sterigmatocystis nigra*. On the contrary it seems that in the flagellate *Dunaliella viridis* the thermal death-point is independent of the amount of NaCl in water, but is much lowered by the presence of Mg (Baas-Becking 1930).

From this standpoint it is important to compare a large number of various salts, in one and the same type of cells, as did Gros (1909), Kaho (1921—1926) and Port (1927*b*).

Gros worked with the red blood corpuscules of a rabbit, which he suspended in isotonic salt solutions and haemolysed by heat at 47.5°—52°. He found that the heat haemolysis was favored by ions in the following order:

$$47.5^0\text{: } Na < Mg < K < Ca, \quad Cl < SO_4$$
$$50.0^0\text{: } Mg < Na < K < Ca, \quad Cl < SO_4$$

At 52.5° the results were approximately the same, but for magnesium salts the sequence of anions was reversed ($SO_4 < Cl$).

Kaho used epidermal cells of various plants which where gradually heated, and the "coagulation temperature" was thus determined. As the rate of heating was uniform in all experiments, the temperature thus arrived at indicates the degree of thermal resistance. In *Zebrina pendula*, coagulation was favored by ions in the following order: Sr, Ba, Mg < Ca; Li, Na < NH_4, K; SO_4 < citrate < acetate, tartrate < $Cl' < NO_3' < J' < Br' < CNS'$.

In general, anions are more effective in depressing the resistance than cations. The series of anions vary somewhat with the nature of the cation (Kaho 1924*b*). The effect of various salts is, according to Kaho (1926), connected with the rapidity of penetration of the respective ions into the cell. Easily penetrating ions depress the resistance much more than do those which penetrate with difficulty.

Port (1927*a*), who studied the same question in *Paramaecium*, reached the same conclusion as Kaho, namely that the effect of ions upon the resistance to heat is connected with their power of penetration into the cell, though the effect seems to be somewhat more complicated in *Paramaecium* than in plant cells (see X. 17).

Nobody has hitherto determined whether salts modify the numerical value of the temperature coefficient of heat injury. Yet it is easy to calculate from the data of Gros (1909) and of Port (1927*b*) that this actually is so. From the figures of Gros

on haemolysis by heat the following values of m (in the PORODKO's formula, see X. 5) are calculated:

TABLE LXXX

Salt	$MgSO_4$	$MgCl_2$	$CaCl_2$	KCl Na_2SO_4	NaCl
m[1])	18.0	23.0	25.2	26.0	28.2

The temperature coefficient (m) increases thus in the following order:

$$Mg < Ca < K < Na; \quad SO_4 < Cl.$$

From PORT's results on *Paramaecium* it appears that the ions increase the value of m in the following series:

Chlorides: $NH_4 < Li < Mg < Sr,\ Ca < Na < K$
Nitrates: $NH_4 < Sr < Ca < Na < Li < K$
Anions: $SO_4 < Cl$

There is here a fair amount of agreement with the results calculated from GROS.

(*d*) *Narcotics.*

According to JOSING (1901) the cessation of protoplasmic streaming in *Vallisneria* by heat is delayed by the addition of 0.125—1.0 % of ether, the most efficient dose being that of 0.25 %. Likewise LEPESCHKIN (1923*d*) reports that the heat resistance of *Spirogyra* is increased by very small doses of narcotics in the beginning of their action. Narcotics with a high dielectric power, as *e. g.* furfurol and nitrobenzol, exert a pronounced protective action.

In the overwhelming majority of cases so far examined there is however a decrease of resistance. This was observed by KOEPPE (1903) for red blood-cells and ethylic alcohol, ether, acetone, chloroform and chloral hydrate (see fig. 63), by WINTERSTEIN (1905) for various animals and ethylic alcohol, by LEPESCHKIN (1911) for epidermal cells of *Tradescantia* and ethylic alcohol, by NOTHMANN-ZUCKERKANDL (1912) for protoplasmic streaming in *Vallisneria* and ethylic alcohol, by HAFFNER (1920) for haemolysis and alcohol, by FRÖHLICH and KREIDL (1921) for marine

1) m is calculated from the periods of time necessary to haemolyse one half of the cells at a definite temperature in the given salt.

crustacea and chloral hydrate and alcohol, by HEILBRUNN (1924*b*) for eggs of *Arbacia* and *Cumingia* and ether, by BĚLEHRÁDEK (1930*b*) and BĚLEHRÁDEK and MELICHAR (1930*b*, 1931) for cells of *Helodea* and chloral hydrate. According to the last metioned authors, chloral hydrate does not affect the duration of life of the sperm of sea-urchin at 27.7°—33.3°, but shortens it at 37.5°—43.8°.

BĚLEHRÁDEK and MELICHAR (1931) also attempted to determine whether the addition of chloral hydrate affects the temperature coefficient of thermal injury. Their experiments on leaves of *Helodea* and on spermatozoa of *Arbacia* showed that this really is so, but the coefficient was decreased in the first case and increased in the other, so that no definite conclusion could be reached. Maybe this is due to the large difference in concentration of chloral hydrate used in the two sets of experiments.

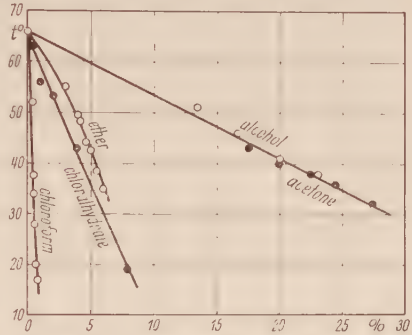

Fig. 63. Action of varied concentration (abscissa) of certain narcotics upon the temperature (ordinate) of instantaneous haemolysis. Constructed from data of KOEPPE 1903.

(e) *Other chemical agents*

It is a well established fact that the thermal sensitivity of bacteria, yeast, bacterial spores, moulds, algae, etc. depends, within certain limits, on the chemical composition of the medium, the effect being due not only to electrolytes, but often to organic substances, like sugars, glycerine, etc. (THIELE 1896, SETCHELL 1903, LEPESCHKIN 1923*d*, AYERS and JOHNSON 1924, OERSKOV 1925*a*, *b*, SHERWOOD and FULMER 1926, WILLIAMS 1928, CURZI 1930, MURRAY and HEADLEE 1931; compare further BUCHANAN and FULMER 1928—1930, vol. II.). It seems that glycerine protects certain types of vegetable cells against heat destruction (THIELE, LEPESCHKIN). It is further known that serum partly protects blood cells against haemolysis by heat (GROS 1909), but this effect is probably due to the partial injury of erythrocytes when they are transported from their normal medium into a

solution of electrolytes with no hydrophilic power, which would balance that of the protoplasm. R. B. Harvey (1924) suggested that the high resistance of thermal algae might be partly due to the presence of CO_2 and H_2S in the medium.

Tolerance to high temperatures is generally lowered by a decrease in the concentration of oxygen, not only in animal tissues (Sanders 1914, A. G. Mayer 1917, Thörner 1920, Libbrecht 1921, Evans 1921, Bělehrádek 1930*b*, etc.), but also in plants (L. Just 1877, Mack 1930). Oxygen is further necessary for recovery after heat-coma in certain cases (Winterstein 1902, 1905, Bondy 1904).

Various alkaloids, hormones, etc., as far as they are toxic, increase the sensitivity to heat (Fröhlich and Kreidl 1921, Barlow and Sollmann 1926). According to Lepeschkin (1923*d*), any injurious chemical agent increases the degree of injury produced by heat.

(*f*) *Mechanical injury*

Lepeschkin (1912) observed that freshly injured or deformed plant cells are less resistant to high temperatures than normal cells, but that they return to normal sensitivity after a certain time. Later the same author (1923*d*, 1924) examined this point more closely and found that mechanical treatment decreases the resistance of filaments of *Spirogyra* to heat. Normal filaments die at 44° in 340 seconds, while filaments which have been bent several times withstand only 100 seconds at the same temperature. A similar result was obtained with red blood corpuscules, normal and agitated.

(*g*) *Osmotic pressure*

From the observations of Schmankewitsch (1877) on crustacea it follows that resistance to heat is decreased when the osmotic pressure of the medium rises. For instance, *Artemia salina*, a crustacean well-adjusted to life in media of highly variable osmotic concentration, dies when the medium is too concentrated, but death may be prevented by lowering the temperature. Similarly *Artemia* is killed by too great a dilution of the medium, but again it is possible to keep the animals alive when the temperature is raised.

The case of crustaceans however is somewhat special because of their ability to withstand important osmotic variations. For

other organisms and single cells the following rule seems to apply, that there is a definite osmotic concentration-point at which the thermal resistance is at its highest, and that resistance decreases on both sides of this point. This appears to follow on the one hand from the fact that hypertonic medium decreases the heat resistance of plant cells (DE VRIES 1871, LEPESCHKIN 1910) and of protozoa (PORT 1927*b*), on the other hand from the fact that a similar decrease of heat resistance is produced by hypotonic media in protozoa (JACOBS 1919), in eggs of sea-urchin (RUNNSTRÖM 1924), and in higher animals (*Convoluta*: GOMPEL and LEGENDRE 1928: *Palaemon*: FRÖHLICH and KREIDL 1921; *Fundulus*: J. LOEB and WASTENEYS 1912).

According to EPHRUSSI and NEUKOMM (1927), there are two osmotic concentrations at which the eggs of *Paracentrotus lividus* show the maximum resistance to a high temperature, namely 23 and 19.5 atmospheres, while the osmotic pressure of the normal sea water is 25 atmospheres. It seems thus that the highest degree of resistance does not always coincide with the natural conditions of the organism.

More recently DÖRING (1932) has found that a dehydration in hypertonic media increases the heat resistance in the ova of *Fucus*.

(*h*) *Radiations*

Heat plus irradiation, acting together, will produce a degree of injury which singly they will not produce. This was proved for ordinary light by KRUSE (1895), THIELE and WOLF (1907), R. WIESNER (1907) and others in bacteria, and by LEPESCHKIN (1923*d*) in higher plants; for ultraviolet rays by BANG (1901), THIELE and WOLF (1907) in bacteria, by BOVIE and KLEIN (1918), BOVIE and DALAND (1923) and FORBES and DALAND (1923) in *Paramaecium*, by POYNTER and MORITZ (1923) in the embryos of *Limnaea*, by MACKEE and ANDREWS (1921) and others in the human skin; for X-rays by RHODENBURG and PRIME (1921) in mouse sarcoma, by DOGNON (1926) in the eggs of *Ascaris*, by HAWKINS and CLARK (1925) in human skin. The same rule applies also to corpuscular radiations, as stated by PAULI and SULGER (1929) and by DOGNON and TSANG (1930) in the case of *Bacillus pyocyaneus*. But it was only in spores of *Bacillus subtilis*, that O. B. WILLIAMS (1928) found that X-rays do not alter the normal response to high temperatures.

Bovie and Klein (1918) demonstrated that exposure to ultraviolet rays actually sensitizes the protoplasm to heat, as there is no cumulative effect when *Paramaecia* are first heated and then irradiated (see also Forbes and Daland 1923). Though the absorbed radiations are partly transformed into heat energy, the combined effect of radiation and heat thus cannot be conceived as simply cumulative. Bovie and Klein have moreover shown that *Paramaecia* may recover relatively quickly from the effect of irradiation and that within 5 hours they lose entirely the sensitivity thus acquired.

An exceptional case is represented by the guard cells of the green plants, in which Leitgeb (1888, quoted from F. Weber 1926*a*), F. Weber (1926*a*) and Döring (1932) demonstrated an increased resistance to heat when they are irradiated by light and when "open", whereas when they are "closed" in darkness, they are less resistant. The adjacent pair of cells behaves just in the opposite way (Weber 1926*b*). The explanation suggested by Weber is that in functioning stomatal cells the pH of the cell-sap is shifted to a value which makes the cytoplasm more thermoresistant, yet Weber emphasises that this is not the only explanation possible, as it is known that light produces important colloidal changes as well.

(*i*) *Effect of density of organisms*

The first observation that an increased number of organisms in a given volume makes them more resistant to heat, is probably due to von Transehe (1913), who worked on Cladocerans. Further evidence is to be found in the bacteriological literature (Bigelow and Esty 1920, H. Weiss 1921, Oerskov 1925*b*, etc.). A systematic study of the phenomenon has been undertaken by Bohn and Drzewina (1926, 1928) in the sperm of the sea-urchin, with an analogous result. A similar observation has been made by W. C. Young (1929*b*) in the spermatozoa of the rabbit and by Jahn (1933) in *Euglena*.

Bohn and Drzewina suggest that "surface catalysis" might play a part in the resistance of cells to extreme temperatures, to variations of osmotic pressure, to poisons, etc., and Oerskov thinks that bacteria form certain protective substances delaying the injury by heat. Yet it is better to leave the question open in the present state of knowledge, especially in view of the

fact that some authors have failed to confirm the phenomenon (Williams 1928 in bacterial spores; Gompel and Legendre 1928 in *Convoluta*).

15. Internal factors modifying the effect of heat

The action of supraoptimal temperatures is often different according to age and other internal conditions of the system. In this respect the action of high temperatures does not differ broadly from that of biokinetic temperatures or extremely low temperatures, whose effect has already been discussed from this standpoint (Chapter III, IV.9, VIII.7, IX.6).

(*a*) *Age*

It will perhaps be advantageous to summarise in tabular form the part played by age in the determination of the resistance to heat (Table LXXXI).

TABLE LXXXI

(Differences in heat sensitivity according to age)

Organism	More sensitive stage	Authority
Bacteriophage	young > old	D'Hérelle 1926
Bacterium coli	young > old	J. H. Schultz and Ritz 1910
Bacillus paratyphi B	young > old	Oerskow 1925*b*
Streptococcus fecalis	young > old	C.N. and P. Stark 1929
Bacterial spores	old > young	Chick 1908
Clostridium botulinum (spores)	old > young	H. Weiss 1921
Bacillus mycoides (spores)	young > old	Magoon 1926*b*
Mosses	old > young	Ewart 1896
Higher plants: leaves	young > very young, buds > old	Sachs 1864*a*
Various plants, nuclei	meristematic > adult	Němec 1909, 1910
Calendula officinalis, root	young > old parts	De Vries 1871
Triticum vulgare, grains	old > young	Munerati 1926
Moina macrocopa (Cladocera)	old > young	Terao and Tanaka 1928
Ancylostoma caninum	larvae > embryos	McCoy 1930
Anthonomus (Insect)	young > old forms	Pierce 1916

Organism	More sensitive stage	Authority
Stegomyia fasciata . . .	larva > imago > pupa > embryo	Macfie 1920
Popillia japonica . . .	larva > embryo > imago > pupa	Ludwig 1928*b*
Culex	larva > embryo, pupa, imago	De Boissezon 1930
Anopheles subpictus . .	pupa > larva	Pruthi 1931
Pseudopleuronectes americanus.	adult > young	Battle 1926
Salmo fario, embryos .	young > older	Andersen 1929*b*, 1931
Rana fusca, embryos. .	young > older	Hertwig 1898
Bufo lentiginosus, embryos.	young > older	King 1903
Anures, larvae.	younger > older	Amerling 1908
Rana butyrrhina	eggs > embryos	Krogh 1914*a*
Rana fusca, tadpoles . .	older > younger	Drzewina and Bohn 1921
Lacerta agilis, embryos .	younger > older	Andersen 1929*a*, 31*b*
Chick oesophagus, rhythm	younger > older	Buglia 1911
amplitude of contraction	older > younger	Buglia 1911
Mouse	young > adult	Stieve 1923

Although it seems that in the majority of cases younger organisms are more sensitive to high temperatures than are more advanced, adult or old organisms, no general rule can be formulated as to the part played by age in the determination of heat tolerance. Such a rule might perhaps be formulated for a limited group of organisms, but certainly not for all organisms as a whole.

(*b*) *Sex.*

From the observations of L. A. Brown (1928) on the rate of killing of Cladocerans by heat it follows that, in this special case at least, sexual differences play no part in the resistance to high temperatures. Yet the point has not been studied enough to enable one to draw a more general conclusion.

The action of heat upon sexual glands and upon their products will be analyzed below (*f*).

(c) *Various vital functions.*

It is a commonly known fact that various physiological processes in one and the same organism are often differently affected by heat. Plant physiologists were the first to observe that photosynthesis is much slowed by heat at temperatures at which the oxygen intake still continues (Schützenberger and Quinquaud 1873). For further details of this phenomenon, repeatedly confirmed in many other vital functions, the reader is referred particularly to the work of Pfeffer (1901—1904) and of Pütter (1911, 1927).

(d) *Regional differences*

It is less generally known that resistance to heat often varies in different regions of one and the same organ even when the cells are nearly alike. Various parts of the heart show a different degree of heat resistance, the ventricle being more susceptible than the auricle and the sinus (Unger 1912, Amsler and Pick 1918, Ishihama 1926). Frog muscles develop heat rigor at a temperature which Vernon (1899) found to be different for various muscles. In the results of this author, however, there is a large margin of error, so that no definite conclusion is possible. But it was suggested by Langendorff and Gerlach (1893) that in the frog the flexors are more easily injured by heat than the extensors. According to Hafemann (1908) the sensitive nervous fibres in the frog sciatic are injured by heat more readilly than the motor fibres. The temperature coefficient of heat injury is also different for different cells (see Table LXXII, p. 174). Ono (1928) demonstrated that various types of white blood cells in the rabbit are killed by 50° after an exposure of varying length:

TABLE LXXXII

	min.		min.
Pseudoeosinophiles . . .	30	Eosinophiles	18
Basophiles	27	Monocytes	14
Small lymphocytes . . .	26	Large lymphocytes . . .	9

F. Weber (1925*b*, 1929, 1932) has recently emphasised that cells of one and the same morphological type are often physiologically unlike owing to differences in the chemical or colloidal constitution of their protoplasm. In fact, when a suspension of

cells of the same type, *e. g.* eggs, spermatozoa, red blood corpuscules, bacteria, protozoa, etc., is exposed to a definite high temperature, they actually do not die all in one and the same moment, but in an orderly succession which has sometimes been expressed mathematically (X. 4). It seems that an analogous rule holds good in multicellular organisms, in whose tissues, too, the single cellular elements do not die at the same time when subjected to a certain degree of heat. This point has been recently studied by Bělehrádek and Melichar (1930*a*) in the leaves of *Helodea canadensis*, in which the wave of injury proceeds from the base

Fig. 64. The "wave of death" proceeding in a definite order from the basis to the apex in heated leaves of *Helodea*. Black space marks the dead cells. Bělehrádek and Melichar 1930*a*.

of the leaf towards its apex, the cells near the axis and at the borders being more rapidly destroyed than the rest (fig. 64). It is noteworthy that the wave of injury by ether seems to proceed exactly in the opposite direction, as has been demonstrated in the same object by Moder (1932).

(*e*) *Malignant growth*

Some investigators were led by practical interests to study the resistance to high temperatures of malignant tumors. Lambert (1912) found that a sarcoma was quickly destroyed by 42.5°, while the normal tissue withstood 46° for 20 minutes. The increased sensitivity of malignant tumors as compared with that of normal tissue was further demonstrated by Westermarck (1927) and Mendel (1928), whereas Friedgood (1928) failed to find any appreciable difference. The paper of Friedgood also contains a list of the earlier literature.

(*f*) *Germ cells*

Various observers have shown that in the most diverse organisms the tissue producing ova or spermatozoa is usually less resistant to heat than the rest of the body. Koch (1911) stated that sexual reproduction in *Hydra fusca* can take place only at 10° and below, though asexual propagation normally occurs at a much higher temperature. It is known that relics of glacial periods, *e. g.* certain species of *Planaria*, etc., do not develop genital glands at a temperature higher than 10° (see Stempell 1926). Likewise in plants the phenomena of reproduction show an optimum which is generally lower than that of the other physiological processes (Klebs 1896; compare Pfeffer 1901—1904). Molliard (1925) found that in *Sterigmatocystis nigra* no conjugation occurs at 36°—44°, although growth still continues.

Many authors have tried to produce thermal castration experimentally in various organisms, and to determine at the same time which of the sexes is more susceptible. The object most studied in this respect is *Drosophila*, in which Northrop (1919) succeeded in producing sterility by heat. His result was confirmed by Plough and Strauss (1923), Young and Plough (1926) and Goldschmidt (1929). The male gonad was found to be less resistant than the ovary. Witschi (1928, 1929) induced a heat degeneration of sexual glands in frog tadpoles (*Rana sylvatica*). The female gland was the less resistant in this case, so that by choosing an appropriate temperature, a regenerative process could be followed by which however testes formed instead of the original ovaries. At 32°, Witschi obtained a stock composed only of males and intersexes, and entirely without typical females. The same author obtained similar results also in *Rana temporaria* (Witschi 1915). As regards mammalian sexual glands, Stieve (1923*a*, *b*), C. R. Moore (1924, 1926), Moore and Quick (1926), Crew (1926), Rostand (1928) and Brummelkamp (1933) found that testes are easily injured by an increased temperature, but according to Stieve (1923*a*), the ovaries of the common mouse seem to be still more susceptible. Stieve also points out that such changes are not necessarily to be interpreted as a primary effect of temperature upon the glands, but that hyperthermia may produce certain alterations of the body which in their turn may modify the gonads.

In *Helix pomatia* exposed daily to 40° for definite periods during several weeks, Bank (1931*a*) obtained a similar degeneration of the gonad which is bisexual in this case. No marked difference between the male and the female part of the gland could be established. Even when the periodic warming was continued, the gonad regenerated spontaneously, yet it assumed a somewhat abormal structure.

The effect of heat on spermatozoa of various animals has often been studied (sea-urchin: J. Loeb 1908*a*, Bohn and Drzewina 1926, 1928, Bělehrádek 1930*b*, Bělehrádek and Melichar 1931; — *Nereis*: Dungay 1913; — mammals: Stigler 1915, 1918, Amantea and Krzyzskowski 1921; Young 1927, 1929 *a*, *b*, etc.). According to J. Loeb (1908 *a*), the spermatozoa of *Strongylocentrotus purpuratus* are more resistant than the ova.

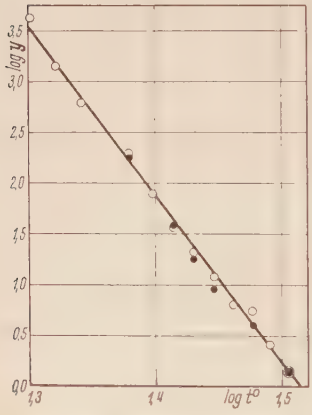

Fig. 65. Mortal exposure ($= y$) to various temperatures ($= t^0$) in fertilized (empty circles) and unfertilized (black circles) ova of the sea-urchin. J. Loeb 1908*a*.

Most studies of the effect of heat upon the egg were performed in the sea-urchin ova, and the literature dealing with this subject has been to some extent discussed above (X. 2, Table LXIX, X. 5 *a*, Table LXXI, X. 6, X. 8, X. 12, X. 13, X. 14 *d*, *g*; further X. 16 *f*). The symptoms accompanying heat-injury are modified by fertilization (Knaffl-Lenz 1908, J. Loeb 1908*a*, Ephrussi and Neukomm 1927, Ephrussi and Parat 1927, Runnström 1928*a*, Tirelli 1930*b*). Injury seems to take place earlier in the fertilized than in the unfertilized ova (Knaffl-Lenz, Runnström, Ephrussi and collaborators), but the exposure and temperature required to cause death seem to be the same in both cases (J. Loeb). Neither is the temperature coefficient of heat injury modified by fertilization (fig. 65). The ability to recover from heat effects in the case of sea-urchin eggs is lost by subsequent fertilization (Tirrelli 1930*b*).

16. Theories of injury by heat

In the following paragraphs we shall review critically the theories hitherto propounded with regard to the mechanism of heat injury.

(*a*) *Coagulation of proteins*

The idea that the effect of heat upon living matter is to produce coagulation of protoplasmic proteins is the most popular of all and seems to be also the oldest. It is not easy to trace back its origins, but as early as 1864 SACHS quoted it as a current view. In the same paper some critical remarks against this view occur, notable among them being that there is a discrepancy between the "coagulation" point of proteins and the thermal death point of the protoplasm. In the years following and in the first decade of this century, the coagulation theory became the dominant view (SEMPER 1880, LEWITH 1890, DAVENPORT 1897—1899, DAVENPORT and CASTLE 1896, THISELTON-DYER 1899, ALCOCK 1903, J. LOEB 1906, 1908*a*, ARRHENIUS 1908, CHICK 1908, BUGLIA 1909, LEPESCHKIN 1910, 1911, 1912, PÜTTER 1914, KANITZ 1915, GROVES 1917, and many others. For recent work compare H. WEISS 1921, BOVIE and DALAND 1923, PRZIBRAM 1923, WURMSER and JAQUOT 1923, LEPESCHKIN 1923*d*, 1924, PORODKO 1926*b*, BROWN and CROZIER 1927, PORT 1927*b*, WILLIAMS 1928; for details of the coagulation theory of heat-rigor in muscle: HALLIBURTON 1887, VINCENT and LEWIS 1901, VROOMAN 1907, v. FÜRTH 1925, etc.). Many authors have spoken of the coagulative change in protoplasm as synonymous with coagulation of protoplasmic proteins, which obviously is not correct.

Among those who criticized the coagulation theory are SACHS (1864*a*), L. JUST (1877), BALLS (1908), MEIGS (1909*b*), A. G. MAYER (1917), ROBERTSON (1920), EVANS (1921), QUAGLIARIELLO (1923), HEILBRUNN (1924*b*), FAURÉ-FREMIET (1925), PFEIFFER (1929), and many others.

Let us consider the similarity between the coagulation of proteins and the heat-injury of protoplasm.

There are no doubt certain changes in the heated protoplasm which may be called "coagulative", among them being the appearance of granules, increase of viscosity and other signs which have been discussed in full in paragraph X. 6. As regards the interpretation of such changes, usually two objections are

raised, namely (*a*) that the temperature at which protoplasm is injured by heat is much lower than that at which proteins coagulate, and (*b*) that practically all the coagulative changes are in the beginning reversible in protoplasm, but irreversible in proteins. Yet it should be noted that, according to certain conceptions, proteins slowly coagulate even at low temperatures and heating only hastens the process (Duclaux 1898). Secondly, proteins may coagulate even at a moderate temperature in the presence of certain electrolytes, this type of coagulation being dependent on temperature as well. At any rate, the difference in this respect between proteins and protoplasm is only a quantitative one. As to the other objection, it must be noted that in certain proteins heat-coagulation is reversible and that the number of observed instances is increasing (Corin and Ansiaux, quoted by Robertson 1909, p. 144; Spiegel-Adolf 1926; Mirsky and Anson 1929; Pfeiffer 1929). Some of these reactions are possibly of a similar type to that described by S. Ringer (1890) in casein, which in the presence of calcium forms at 70° a coagulum which is perfectly reversible on cooling. Gray (1931) has recently claimed that such processes might take place in the protoplasm on heating, yet only when the reaction is alkaline.

The numerical value of the temperature coefficient is unusually high, both in the case of injury by heat and in the coagulation of proteins. This striking analogy was pointed out by Arrhenius (1908, 1915), J. Loeb (1908*a*), H. Chick (1908, 1910), Buglia (1909), Lepeschkin (1912), Kanitz (1915) and many others. There are however certain other chemical reactions which yield a similarly high temperature coefficient, *e. g.* action of hot water upon starch grains, which is connected with a $Q_{10} = 57—83{,}900.000$ (Lepeschkin 1922*b*, 1923*a*).

Low water content increases the resistance of proteins to heat (the older literature is quoted in Davenport and Castle 1896; compare further Chick and Martin 1910, 1912*b*, Lepeschkin 1924, Lecomte du Noüy 1930, etc.). Absolutely dry protein cannot be coagulated at all. Similarly it is known that a decrease in water content of living systems increases their resistance to heat, as has been demonstrated above (X. 14*a*). When however a living cell is dehydrated osmotically, the reverse effect is usually produced (X. 14*g*).

It is commonly known that the change produced by heat in proteins involves two separate processes, namely (*a*) denaturation, a kind of hydrolysis, which cannot therefore take place in the absence of water, and gives a very high Q_{10}; and (*b*) coagulation, agglutination or flocculation, which is a physical surface-phenomenon, which requires the presence of salts and whose Q_{10} is rather low. A dialysed solution of protein does not coagulate on boiling, but once boiled, it coagulates at a normal temperature on subsequent addition of electrolytes. For details the reader is referred to papers of Chick and Martin (1910—1912) and of Lepeschkin (1922—1923), in which also the earlier work is discussed. The participation of salts in the process varies not only with their amount and with their chemical nature, but also with the pH. At the isoelectric point the addition of salts does not seem to modify the temperature at which proteins begin to coagulate on gradual heating (Kakiuchi and Koganei 1922, J. H. Clark 1925*a*, *b*, M. and S. P. L. Sörensen 1925). In acid reaction the salts accelerate coagulation, according to Spohr (1888), in the following order:

$$SO_4 < Cl < Br < SCN$$

For the physical coagulation alone, Lepeschkin (1923*c*) recorded the following series:

$$SCN < J < Br < Cl < \text{tartrate} < SO_4,$$
$$K < NH_4 < Na < Li;\ Mg < Ba;\ Al < Fe$$

When these data are compared with what is known on the action of salts in the heat injury of protoplasm (X. 14*c*), it is evident that these phenomena do not lend much support to the coagulation theory.

When the proportion of salts remains unchanged, the rule holds that proteins coagulate more easily when acid than when alkaline, the maximum of stability coinciding with the isoelectric point (Chick and Martin 1910—1912; Lepeschkin 1922—1923; Kakiuchi and Koganei 1922; J. H. Clark 1925; Sörensen and Sörensen 1925, etc.). Living cells seem to behave in a similar way, as has been shown above (X. 14*b*). Yet the experiments all deal with the pH of the medium only, and not with that of protoplasm.

Narcotics lower resistance to heat both in proteins (Lepeschkin 1910, 1924) and in protoplasm (see X. 14*d*), yet there seem

to exist two important differences. First there are certain narcotics which, on the contrary, increase the resistance of the protoplasm to heat (Lepeschkin 1923*d*), and secondly narcotics in general do not seem to modify the numerical value of the Q_{10} in the case of coagulation of proteins (Lepeschkin 1924), but do modify it in the case of heat injury of living cells (see X. 14*d*).

Bovie (1913) demonstrated that egg albumen is more easily coagulated by heat when previously irradiated by short rays. Similarly in living cells the action of heat is often increased after a previous irradiation, as we have already seen (X. 14*h*). But J. H. Clark (1925*a*, *b*) has recently observed that proteins are sensitized to heat only after irradiation in an acid medium, whereas irradiation in an alkaline medium rather protects the protein against the subsequent action of heat.

According to known facts, we may now conclude that the coagulation theory cannot altogether explain the mechanism of heat injury. Although death by heat at very high temperatures undoubtedly is a coagulation, it should be remembered that protoplasm contains other substances besides proteins, and that injury by heat involves many changes which, especially at moderately high temperatures, cannot be conceived of as coagulative.

(*b*) *Heat destruction of enzymes*

Tammann (1895), Duclaux (1898—1899) and many later researchers demonstrated that enzymes *in vitro*, too, possess an optimum temperature of activity and are destroyed at a temperature which is usually close to the temperature maximum of living organisms. F. F. Blackman (1905) applied these facts to biology and was followed by numerous other workers (Jost 1906, Kuijper 1910, 1911, Rutgers 1911, E. N. Harvey 1911, Arrhenius 1915, Robertson 1920, etc.). Among those who raised objections against this idea were Cohen-Stuart (1911), van Amstel and Iterson (1911*a*), Kanitz (1915), Groves (1917) and others (compare further II. 5).

The most striking analogy between enzymes and protoplasm is undoubtedly their temperature optimum, beyond which the activity rapidly sinks. It must be however noted that many simple chemical reactions possess an optimum as well (Errera 1896; Taylor, quoted by E. N. Harvey 1911, Kanitz 1915, Stern 1925). Recently Nicloux (1928) found a typical optimum

curve in the spontaneous oxidation of glucose (fig. 66). It has been further pointed out by PHILIP (1915) that colloidal systems in general must possess an optimum.

Heating actually decreases the amount of enzymes in seeds, as was demonstrated by LANTZ (1927). D'AVANZO (1929) on the contrary found an increased percentage of certain enzymes in the mammalian skin, heated to 45°—46°. On the other hand it is easy to demonstrate that in dead tissues active enzymes still are present (GROVES 1917).

The heat destruction of enzymes yields a high temperature coefficient and is retarded by exsiccation. In these two respects it closely resembles the destruction of protoplasm by heat, yet with certain restrictions which are analogous to those mentioned

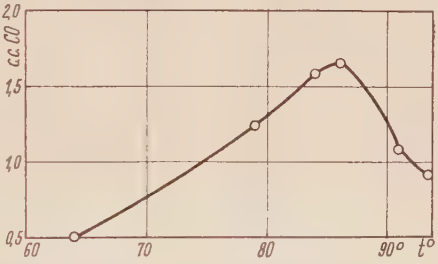

Fig. 66. Spontaneous oxidation of glucose in an alkaline medium, according to NICLOUX 1928. Abscissa: temperature. Ordinate: amount of CO produced per unit of time. — Note the optimum in the vicinity of 85°.

above in connection with coagulation of proteins (X. 16*a*). A further similarity between protoplasm and enzymes is that the optimum decreases with increasing time of observation (X. 1, X. 4: for enzymes, compare DUCLAUX 1898-1899, ARRHENIUS 1915, BERTRAND and COMPTON 1921, BERRILL 1919, PANTIN 1932 etc.).

In all other respects however there exist important differences between the destruction of enzymes and of the protoplasm by heat.

The sensitivity of enzymes to high temperatures is modified by the presence of salts, as was first demonstrated by VERNON (1901) and later by many others. Yet no comparison with salt action upon the thermal resistance of the protoplasm can be made, the results being widely different in enzymes and in protoplasm. Certain salts protect enzymes against heat-injury, while in living systems such cases are extremely rare.

Likewise there are important differences in the action of hydrogen ions on the heat destruction of enzymes on the one hand,

and upon that of protoplasm on the other. Enzymes usually show a maximum resistance to heat at a definite pH, which is often far below or above the optimum pH of activity (Ernström 1922, 1923, Sjöberg 1922, Pincussen and Oya 1929, etc.), while the protoplasm is most thermo-resistant at a pH which more or less coincides with the neutrality point (see p. 192). The temperature coefficient of the destruction of enzymes by heat often depends on pH (Euler and Laurin 1919, Sjöberg 1922, etc.), but no serious comparison is possible with the analogous phenomenon in living cells (X. 14*b*), because in the latter case only the external pH is known.

Ethyl alcohol accelerates the destruction of emulsine by heat (Olive 1931), the medium concentrations (30 %—70 %) being more efficient than the low and high ones. It is evident here also that the enzyme differs greatly from the protoplasm (X. 14*d*).

Although opinions on the reversibility of the heat inactivation of enzymes are largely at variance, it seems that under certain conditions a partial reversibility is possible, especially when the destruction is not too advanced (Miyake and Ito 1923—1924, Northrop 1932). At any rate the reversibility is much slower and more incomplete in enzymes than in protoplasm.

It may thus be concluded that present knowledge lends only little support to the idea that the heat-destruction of enzymes is the primary cause of injury of living systems by heat. Furthermore, the notion does not explain such symptoms of heat-effect which have nothing to do with enzymic action, *e. g.* change in permeability.

For a more detailed documentation of the discussed properties of enzymes the reader is referred to special monographs by Bayliss (1925), Kuhn (1924), Oppenheimer (1924), E. Stern (1925), Haldane (1930), etc.

(*c*) *Asphyxiation theory*

The effect of high temperatures is similar to the effect of absence of oxygen in that, after a short period of excitation, it paralyses the vital activity first temporarily and then permanently. This analogy, which of course should be extended to narcotics as well, led Winterstein (1902, 1905) to formulate the hypothesis that injury by heat is caused by asphyxiation. According to this

view, rising temperature increases metabolism and thus the need of oxygen, but the oxygen supply can no longer keep pace with the demand chiefly because owing to warming, the power of the medium to absorb oxygen is decreased. This view, shared by Verworn (1914) and others, is supported by certain facts. First we have already seen (X. 14) that the absence of oxygen actually lowers the resistance to heat in many living systems. Secondly it has been demonstrated by Winterstein (1902, 1905) and Bondy (1904) that oxygen is necessary to the process of recovery following overheating.

Yet there are also certain facts which do not lend support to Winterstein's hypothesis. Anoxybiotic organisms are killed by heat almost as easily as oxybiotic organisms. The heart of *Limulus* beats for 12 hours and more in the absence of O_2, but it is quickly paralysed by a temperature of 32°—42°, as was demonstrated by Carlson (1906). Babák and his pupils were the first to bring together certain observations in opposition to the asphyxiation theory. *Rana fusca* is more resistant to the absence of oxygen than *R. esculenta*, but the former is paralysed within three minutes at 35°, the latter at 40° (Babák 1907). In tadpoles of *R. esculenta* and of other anurans sensitivity to lack of oxygen increases with age, whereas sensitivity to heat decreases (Amerling 1908; see further Babák and Roček 1909). Löhner (1913) studied the resistance to various agents in ciliates, which he ranged in series according to their increasing sensitivity. These series are widely different for resistance to heat, asphyxiation and narcosis.

Even if the asphyxiation theory cannot interpret all the phenomena of heat injury, it must be conceded that in a low oxygen tension, and in living systems with a high demand of oxygen, moderately high temperatures produce paralysis or death by asphyxia. For other cases, however, asphyxia is not the determining agent, because death is produced by some other factors before asphyxia can develop.

(*d*) *Intoxication theory*

The fact that heating accelerates various chemical processes to a different degree, and that equilibria in general are modified by temperature, has led some investigators to postulate that similar changes, which possibly occur in protoplasm at higher

temperatures, are responsible for heat injury. This idea has once been formulated as the assumed change in protoplasmic equilibria (Sachs 1864*a*, Demoll and Strohl 1909, Goldschmidt 1920, Bovie and Daland 1923, Fauré-Fremiet 1925, Höber 1926, Löhner 1930 etc.), again, as the assumed poisoning of the system by changed metabolic products, chiefly acids, or by a change in concentration of the normal metabolic products (Balls 1908, Meigs 1909*a*, *b*, A. G. Mayer 1917, etc.).

Such changes actually take place, as has been sufficiently demonstrated (X. 10). Yet the question again is whether such changes can be made responsible for heat injury in general. In considering that at very high temperatures the system is destroyed with a velocity which would scarcely allow of an accumulation of metabolic products up to the toxic effect, the theory is probably valid only in the zone of moderately high temperatures, as in the case of the asphyxiation theory. There is a further analogy between these two ideas, namely that asphyxia itself leads to an accumulation of acid products in the protoplasm. The fact that a decrease of activity may often be observed before any structural changes take place in the cell (X. 11), speaks again in favor of these theories.

(*e*) *Lipoid liberation theory*

The increasing degree of importance attributed to lipoids in the constitution of cells gave rise to the idea that the effect of heat upon protoplasm is due to some change in the physical state of lipoids. Koeppe (1903) interpreted haemolysis from heat as a "melting" of lipoids contained in the superficial layer of red blood-corpuscules. Rywosch (1911), like Koeppe, spoke of the "melting point" of erythrocytes and studied its relation to the action of lipotropic substances, *e. g.* saponin. H. Meyer (1909) attributed to lipoids a similar part in the determination of heat-coma. The idea was recently generalized by Heilbrunn (1924*b*, 1928) and Fauré-Fremiet (1924, 1925), who showed that exposure to supraoptimal temperatures actually produces changes in the lipoid constituents of protoplasm. According to Lepeschkin (1924, p. 167), lipoids are necessarily liberated from their combination with proteins, when the latter begin to undergo denaturation by heat. Similarly Fauré-Fremiet (1924, 1925) maintains that the protoplasm is a heterogeneous system in metastabile

state, *i. e.* in a state of untrue equilibrium, which passes into a true equilibrium on heating beyond a "critical" temperature. — These concepts, which are the result of modern experimental study, recall those of SACHS, who in 1864*a* supposed that the normal properties of living matter are based on equilibria between multiple "molecular forces", as he expressed it, and these are broken by any external force of sufficient strength, *e. g.* by a high temperature.

Loss of permeability by heat, heat-haemolysis and especially certain directly observed changes in lipoid constituents of protoplasm lend much support to the lipoid theory. All these phenomena have been analysed in detail above (X. 6*f*, *g*, X. 9) and need not be discussed here. The action of narcotics in conjunction with heat has been claimed by KOEPPE (1903) and more recently by HEILBRUNN (1920, 1924*b*) in favor of the lipoid theory, but this argument loses much of its weight by the fact that *e. g.* ethylic alcohol accelerates not only the heat coagulation of protoplasm, but also that of proteins and enzymes (see above, X. 16*a*, *b*). The strongest support to the theory, however, is provided by the fact that there is a clear-cut relation between the heat resistance of organisms and the melting-point of their protoplasmic fats and phosphatids. This point, emphasised by RYWOSCH (1911), HEILBRUNN (1924*b*) and BĚLEHRÁDEK (1931*a*), will be discussed in the next paragraph in connection with the mechanism of thermal adjustment.

(*f*) *Relative value of the theories discussed*

With the advancing study of heat injury it becomes more and more obvious that the effects of high temperatures upon living systems are so complex that no theory could venture to reduce them all to a uniform basis. It must be assumed that any of the mechanisms suggested by the theories just discussed, may become under certain conditions the primary factor in the determination of death by heat. In this respect the action of heat is somewhat analogous to the action of frost (VIII. 4). It will not be out of place to give here some facts which seem to lead to such a view.

It has often been observed that the morphological and other changes produced by heat-injury are different according to the temperature applied. KNAFFL-LENZ (1908) observed that in the

egg of the sea-urchin swelling and cytolysis occur at 42°—52°, while on the application of higher temperatures the eggs coagulate without changing in volume. The same phenomena have been studied more recently by Runnström (1924, 1928*a*, *b*) and Hörstadius (1925) on the same object, and by Hörstadius (1923) on the eggs of an annelid, *Pomatoceros triqueter*. It seems that the temperature above which the cytoplasm coagulates, is sensibly higher in unfertilized eggs (Runnström). It is similarly known that heat hemolysis occurs at not very high temperatures, while at very high temperatures haemoglobin has no time to escape because of rapid coagulation. When an isolated muscle is gradually heated, several contractures develop at definite temperatures which, according to earlier researchers, agree with the coagulation temperatures of proteins extracted from the muscle. It was however demonstrated by Scaffidi (1913) that no such agreement between the two coagulation temperatures actually exists, and that the successive heat contractions are probably caused by different processes.

As has been pointed out by Bělehrádek and Melichar (1930*a*, 1931), the temperature coefficient of the length of survival in various living systems is low for moderately high temperatures and higher for very high temperatures, and there is often a break in the temperature-time graphs (see fig. 67) in the neighbourhood of 35°—45°. Hence the conclusion of the authors that at least two separate processes are the cause of injury by heat at various temperature intervals. We have seen in Chapter IV (IV.3, IV. 4), how dangerous it would be to draw similar conclusions from the mere external aspect of graphs and their respective constants. The fact that the same differences in temperature coefficient and the same "breaks" are obtained with any of the current formulae discussed in X.5, lends some support to the view of the authors, which without the morphological observation mentioned above and without additional experiments on the part of the authors (1930*b*, 1931) on the combined action of heat and narcotics upon animal and plant cells would of itself appear rather vague. These experiments, already quoted in another connection (X. 14*d*), demonstrated that the action of chloral hydrate is actually different at various ranges of temperature, as regards the depression of the resistance to heat and the numerical value of the temperature coefficient. Likewise Port's (1927*b*) results on *Paramaecium*

seem to support such views. This author found that salts, which permeate slowly into the cell, accelerate death by heating below 40°, but retard it above this temperature.

More data will be necessary before the part played by various processes which are assumed by the enumerated (*a*—*e*) theories, can be formulated in a clearer and more general way.

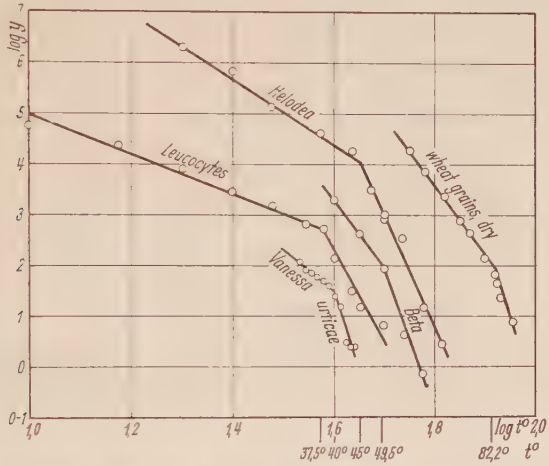

Fig. 67. Several examples of "breaks" in the time-temperature graphs drawn as required by Porodko's equation. Constructed from data of Prochnow 1914 (pupae of *Vanessa urticae*), Ono 1928 (mammalian leucocytes), Collander 1924 (*Beta*), Bělehrádek and Melichar 1931 (*Helodea*) and Groves 1917 (dry wheat grains).

17. Thermal adaptation

Apart from organisms which are exceptionally heat-resistant in a dry or semi-dry state and in which resistance is probably maintained chiefly by the low water content (X. 14*a*), there are numerous differences in the resistance of organisms in a normal state. Such cases obviously cannot be understood in the same way, neither can it be admitted generally that the differences in resistance are due to differences in the composition of the medium, though this factor is sometimes important (X. 14*b*—*e*).

It is known that unicellular organisms may become adapted to an abnormally high temperature, as was demonstrated by the well-known experiments of Dallinger (1887) on flagellates, and

by numerous culture experiments on bacteria (see, *e.g.*, Dieudonné 1894, Pfeffer 1901—1904, Magoon 1926, Williams 1928, etc.). Likewise *Paramaecia* are able to adapt themselves to heat (Mendelssohn 1895, Jacobs 1919, Michelson 1928). Isolated organs, too, sometimes present signs of an adaptive change which often takes place during the experiment itself (frog nerve: Sanders 1914, Thörner 1920; human leucocytes: Madsen, Wulff and Watabiki 1919). It is less surprising that whole higher organisms often modify their resistance to heat when gradually accustomed to it. This was demonstrated by Davenport and Castle (1896) in tadpoles, by J. Loeb and Wasteneys (1912) in *Fundulus*, by Schaefer (1921) in certain medusae, by Sayle (1928*a*, *b*) in certain insects, etc.

In some cases thermoresistant organisms appear at once as new mutants. Thus Hance (1915, quoted from Jacobs 1919) obtained a stock of *Paramaecium caudatum*, which differred from the normal breed by having three contractile vacuoles instead of two, and by an increased resistance to high temperatures. Wood and Banta (1926), Banta and Wood (1927) demonstrated a new thermoresistant and thermophilic mutation of *Daphnia longispina*. Richet, Bachrach and Cardot (1925) suggested that the presence of KCl produces a hereditary increase of the temperature optimum in *Bacillus acidi lactici*.

In some isolated instances resistance to heat seems to be associated with the special biochemical constitution of the organism concerned. Thus thermal algae are characterised by the lack of catalase (R. B. Harvey 1924). Thermal bacteria without chlorophyll are more resistant than the thermal green algae, as pointed out by Setchell (1903), and the same difference seems to exist between the green and colourless parts of higher plants (de Vries 1871). Yet observations on such a limited group of organisms cannot serve as a basis for more general speculation.

The general cause of resistance to heat has been looked for in various properties of protoplasm. Extant literature on this subject has been recently summed up by Bělehrádek (1931*a*) and it will suffice here to give a succinct extract.

It appears that in organisms in an active state there is no simple relationship between thermal resistance and the amount of water in the protoplasm. Higher temperatures tend to depress

the degree of hydration of living systems, but it is not certain whether such an effect is associated with an increased resistance. Earlier results on coagulation of proteins seemed to show that the coagulation temperature of proteins is parallel to the thermal death-point of a given organism, but the more systematic studies of Kryż (1907) demonstrated that this is not the case in reality. The question whether enzymes adapt themselves to a change in temperature, has been answered in the negative by the recent investigations of Luippold (1929, compare further Pantin 1932).

Among the general chemical constituents of living matter only fats and phosphatids undergo important changes when the temperature at which they are produced is varied. We have discussed this point closely in Chapter V (V.3), to which the reader is referred for details and figures. It is a general rule, now well established for various lipoids of animal and vegetable origin, and supported also by thermodynamic arguments, that the melting point of fats and phosphatids varies in accordance with the temperature at which they have been formed. Rywosch (1911), experimenting on various mammals, established a definite relationship between the sensitivity of red blood cells to heat and the melting point of the fats. The higher the melting point of the fats, the higher the temperature of instantaneous haemolysis. Heilbrunn (1924*b*) and Bělehrádek (1931*a*) have enlarged this idea upon the thermal resistance of organisms in general, and suggest that the melting point of the protoplasmic lipoids determines the resistance to heat. This applies however only if moderately high temperatures are involved which do not cause coagulation of proteins. The most important results in regard to this theory are those of Terroine, Hatterer and Roehrig (1930), which showed definitely that temperature modifies the chemical constitution of protoplasmic fats and phosphatids, and not only that of reserve fats. The fact that the temperature coefficient of vital functions in general increases with the temperature to which the species in question is adapted, lends further support to this view. This point has already been discussed in paragraphs III. 15 and IV. 9*i*, where the probable explanation is given, namely that increased temperature, at which the organism develops, produces an increase of the melting point of lipoids, resulting in an increase of their consistency and a

consequent increase in protoplasmic viscosity. Likewise the phenomena of chilling (IX. 8) may be interpreted on similar lines. Thus the theory of the participation of lipoids in the action of temperature in general connects the following phenomena: (1) chilling, (2) heat injury, (3) heat resistance, (4) increase of temperature coefficients of vital functions with thermal adaptation, and (5) modification of chemical constitution of lipoids with temperature.

CHAPTER XI

STIMULATIVE EFFECTS OF TEMPERATURE

1. General

Apart from the phenomena analyzed in the foregoing sections of this monograph, there are many other types of biological temperature actions which are commonly denoted as stimulative. Thus organisms performing thermotactic, thermotropic and other movements are excited by temperature. Seeds may also be induced to germinate by temperature variations. Sudden changes of temperature may produce temporary inhibition of protoplasmic streaming, heart-beat, etc.

Although one understands the difference existing between such phenomena and the other kinds of temperature action previously discussed, it is extremely difficult to draw a sharp line between direct and stimulative effect of temperature. Certain authors (*e.g.* Lefèvre 1911, p. 243, Broemser 1927) consider every change produced by temperature as stimulative, yet it seems preferable to reserve the term to phenomena which occur as a consequence of a temperature change, usually rapid, and which are not primarily dependent upon the degree of temperature applied, and cannot thus be predicted as the simple acceleration or retardation of vital processes due to rising or falling temperature. These phenomena must be dealt with together with the stimulating effect of short exposures to extreme temperatures.

2. Protoplasmic streaming

Temporary acceleration, slowing or cessation of the streaming of cytoplasm in plants with a sudden change in temperature was first described by Dutrochet (1837) and later confirmed by Hofmeister (1867), H. de Vries (1870), Hauptfleisch (1892), Klemm (1895), Hörmann (1898), Josing (1901), Ewart (1903) and

others. Velten (1876) failed to observe such an effect in *Helodea* and *Vallisneria*, although Hauptfleisch and Josing observed this in the same objects. More recent studies of the phenomenon are those of Lambers (1925), S. F. Cook (1929) and Romijn (1931).

The intensity of the effect produced depends on many variables, in the first place the extent and rapidity of the temperature change (Hauptfleisch, S. F. Cook). Both raising or lowering of temperature in certain cases produces the same effect, as was described by Hauptfleisch, but in other cases the effect is not the same with sudden warming as with sudden cooling the

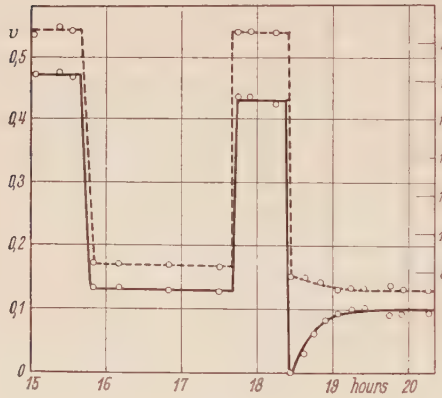

Fig. 68. Velocity (v) of protoplasmic streaming in *Nitella* subjected to variations in temperature. Abscissa: time. Ordinate: velocity (left side) and temperature (right side). Full line: velocity of streaming. Dash-line: temperature. — Note the course of the spontaneous return to streaming after a complete cessation at 18 hrs. 26 min. (Bělehrádek).

same number of degrees. Thus Josing observed in *Vallisneria* a temporary acceleration of protoplasmic streaming on cooling from 20° to 6°, and a retardation on heating from 6° to 20°. On the other hand, Hörmann and Romijn saw in *Helodea* and in *Nitella* respectively that over the same range of temperature, cooling was more effective than warming.

As to the sequence of these phenomena, Josing observed that in *Vallisneria* a change from 1.5°—3.0° to 20° produces first complete cessation, followed by a short period of slow streaming, then after 15 minutes a period of increased velocity, which after further 15—20 minutes was again decreased. The velocity returns to its normal only after 6 hours. The hitherto unpublished data of Bělehrádek (Fig. 68) however indicate a much simpler course, and Lambers, who worked with *Nitella mucronata*, likewise observed a return to normal velocity within 10—20 minutes.

S. F. Cook, working with *Nitella clavata*, demonstrated that the effect is independent of the temperature attained, but depends on the extent of temperature change. The effective exposure to cold is surprisingly short, only half a second being required to arrest streaming in cells cooled from 35° to 10°. The return of streaming is much slower and it demands a longer interval of time, the shorter the exposure to cold. This is explained by the assumption of the author that the recovery process starts at the temperature at which cessation took place. The temperature coefficient of this process is low ($Q_{10} = 1.45—1.51$).

The intimate mechanism of the phenomena discussed here is not known and it must be supposed that it is the same as the

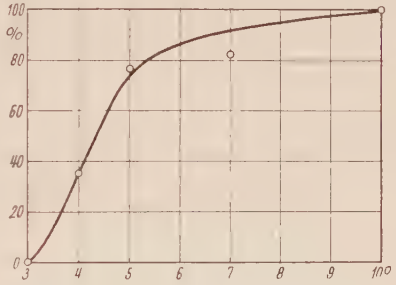

Fig. 69. Percentage of cells (ordinate) of *Nitella* which have remained unaffected by a sudden transfer to a low temperature (abscissa). Constructed from data of S. F. Cook 1929.

mechanism of excitability in general. In support of this view, Umrath (1930) has found in *Nitella mucronata* that an electropositive wave originates on sudden cooling and that the potential returns slowly to the normal value when the cells are subsequently warmed. The phenomenon takes place even on slower temperature changes and is polyphasic (Umrath 1934). Josing (1901) observed that the addition of ether depresses the sensitivity of protoplasm towards sudden temperature changes.

Individual differences between single cells have been studied by S. F. Cook (1929) in *Nitella clavata*. His results are summarised by the following figures:

TABLE LXXXIII

Temperature to which the cells were transferred from 20°	10°	7°	5°	4°	3°
% of affected cells.	0	18	23	65	100

The graphical representation of these figures (fig. 69) forms a sigmoid curve rather than a simple one as drawn by Cook,

because in such cases the sigmoid graph is another expression of the normal curve of variability (see X. 4).

In many other types of vegetable cells which do not show cytoplasmic streaming, temperature stimulation is known to produce protoplasmic nodosities, clumps, protrusions, etc. (See LUNDEGÅRDH 1922, p. 267.)

3. Other motor functions

Zoospores of various algae are temporarily immobilized by a sudden variation of temperature, as was first demonstrated by STRASSBURGER (1878). A sudden cooling produces, according to PÜTTER (1900), a temporary acceleration of the ciliary movement in *Stylonychia*. Frog muscle contracts with any sudden thermic change and JENSEN (1909) thinks that all contractile substances react in the same way.

The rhythmical movements of higher organisms are often equally sensitive to rapid variations of temperature. Thus E. N. HARVEY (1911) observed a reversible acceleration of pulsations in the medusa, *Cassiopea xamachana*, and since CYON's (1866) researches it has been known that a frog's heart reacts to sudden cooling by irregular or wormlike movements, accompanied by arhythmia or even ending in a reversible cessation. The similar anomalous response of the heart to temperature was often described as the "thermal paradox" of the heart and may sometimes attain such a degree as to make it quite impossible to study the temperature coefficient of the heart-beat. For details of this special point the reader is referred to the papers of PLATEAU (1880), KOCH (1917), LIBBRECHT (1921), GARTKIEWICZ (1926), MANGOLD (1926), POTONIÉ (1926), MAC ARTHUR and BAILLIE (1929*b*). Similar irregularities of rhythm, due to sudden cooling, were demonstrated in pieces from the arteries of the calf by CHRIST (1929). It is however to be noted that in organs which possess nervous elements, as *e. g.* the heart, the effect of sudden temperature changes upon the rhythm may be secondary. The same seems to apply to the contraction of chromatophores in certain fishes on rapid changes of temperature. This phenomenon has been recently studied by D. C. SMITH (1928), whose paper includes a review of the earlier literature.

As to the motor functions of plants, WALLACE (1931) noticed a reversible checking of reactivity in *Mimosa* on sudden cooling

or heating within the biokinetic zone. It is further known from the investigations of Senn (1908) that extreme temperature variations cause a reversible contraction of chloroplasts in plants.

4. Nervous system

The various thermo-sensory organs in various organisms, and the mechanism of their action, lie outside the purpose of this monograph and cannot be discudded here. Suffice it to note here that, apart from such special nervous organs, the nerves in general are excitable by temperature variations, when these are rapid enough. Rosenthal (1864) noticed muscular twitches in the region supplied by the ulnar nerve in man, when the elbow was suddenly immersed into ice-water. For further information special articles should be consulted (Broemser 1927).

5. Thermotropism

It would also lie outside the scope of this monograph to discuss here the mechanism of thermotropic response. There appeared recently, moreover, a special treatise devoted to the subject (M. Rose 1929). Still it may be of more general interest to know that the discriminating power of the thermotropic organisms may be very high. Mendelssohn (1895) estimated that *Paramaecium* can react to a difference of only 0.01^0 between the two opposite sides of its body.

6. Stimulation of metabolism

The stimulating effect of variations in temperature has been often observed in diverse metabolic processes. The production of oxygen in plants is temporarily arrested after a sudden transfer from the cold to room temperature, as was found by Ewart (1896) and more recently by Beljakoff (1930). Similarly the rate of respiration in plants is temporarily increased under such conditions (Palladin 1899, Zaleski, quoted by Iraklinow 1912; E. F. Hopkins 1924, Boresch 1928, Hée 1929). In animals such thermic stimulation of metabolism has been rarely observed and when it occurs, it may be due to some nervous mechanism. Yet Baudin (1932) recently found that sudden temperature variations stimulate O_2-consumption in the gold fish, *Carassius auratus*, and that deep narcosis does not abolish the response.

7. Bioelectric currents

We have mentioned above (XI.2) that a stimulation of *Nitella* by a rapid or even slow temperature change gives rise to an action current (Umrath 1930, 1934). Likewise Lund and Moorman (1931) have observed an increase of potential difference in frog-skin immediately after a change in temperature.

8. Thermic parthenogenesis

Morgan (1900) was first to observe that sea-urchin eggs may be activated by a low temperature, and Delage (1901) found the same for supraoptimal temperatures. According to Greeley (1902*c*, 1903*a*), an exposure to 4°—7° for several hours brings about activation in the eggs of the starfish (*Asterias forbesii*). There seems to be an optimal length of exposure, outside which the percentage of activated ova rapidly falls (fig. 70). On the other hand, R. S. Lillie (1908) obtained artificial parthenogenesis in the same organism on application of 35°—38° for 20—70 minutes. With a higher temperature a shorter exposure was sufficient. Lillie's data agree well with the formula of Porodko (X.5*d*) and yield an $m = 14.8$, *i.e.* of the same magnitude as in heat-injury (see X.5). That the tendency to thermic parthenogenesis is not restricted to the group of echinoderms, was demonstrated by Just (1915), who obtained analogous results in an annelid, *Nereis virens*, and by Koltzoff (1932), who induced parthenogenesis by heat in ova of *Bombyx mori*.

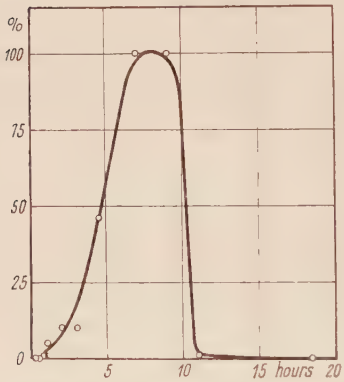

Fig. 70. Variation in the sensitivity of eggs of a starfish to a low temperature (+4°), according to Greeley 1902*c*. Abscissa: exposure to cold. Ordinate: percentage of ova activated by that exposure.

Certain types of activation, which perhaps may be compared with parthenogenesis, occur likewise in plants on application of higher temperatures. Thus Klebs (1896) succeeded in activating oogonia of *Vaucheria* by heat and the same author observed that the zoospores of *Protosiphon*, which otherwise conjugate, ger-

minated without conjugation at 25°—27°. Similarly NATHANSOHN (1900) described a spontaneous segmentation of ova in *Marsilia* when exposed to a supraoptimal temperature. According to SHIBATA (1902), endospermic nuclei begin to divide in *Monotropa uniflora* on application of slightly supraoptimal temperatures (28°).

9. Activation of other reproductory processes

It was observed by GREELEY (1902*a*) that *Stentor*, kept at 25°—28°, proceeded to divide rapidly before dying. When the animals were transferred to a lower temperature, the process of division, even when it had begun, was reversed. The same author (1902*b*) described a division with simultaneous formation of spores in *Monas* when kept for several days at +1°. That rapid changes of temperature induce formation of cysts, is sufficiently known from the researches of R. HERTWIG (1903), E. F. ADOLPH (1929) and others.

Fertilized eggs of certain animals do not develop unless previously cooled on ice for a definite period. This is known for the eggs of *Branchipus* (BRAUER 1877) and for the winter eggs of *Apus* (see GREELEY 1903*a*). Similarly the statoblasts of certain bryozoa behave in an analogous way. Likewise the spores of ferns may be activated by heating them to 32° (DE FOREST-HEALD 1897).

ARENS (1929*a*) reports that the previous cooling of the oospores of *Plasmopara viticola*, a phycomycete, to below 0° accelerates the subsequent development.

Yet the best studied case of thermic activation is that of seeds, first demonstrated by L. JUST (1877), who observed a favorable effect of heat (60°—70°) upon the subsequent germination of many types of grains. EIDAM (1883), CIESLAR (1883), v. LIEBENBERG (1884) and others later observed that the germinating power of various seeds is greatly increased by alternatively cooling and warming them. KINZEL (1911) noticed that certain grains, which could not germinate for years, did germinate very soon when they were subjected to the action of frost. It is now generally known that many seeds require a temporary lowering of temperature to be able to germinate subsequently, and in many other types of seeds such a treatment at least greatly favors germination. DOERFEL (1930) found that the efficient agent is not the frost itself, but any temperature change of a sufficient

extent, and that the temperature does not even need to sink below 0°. There is now a vast bibliography on the subject (Kinzel 1911, Harrington 1919, Munerati 1920, Morinaga 1926, Lute 1927, Pojarkova 1928, H. C. Joseph 1929, Busse 1930, Gassner 1930, Papadakis 1933, etc.).

Likewise it is possible to bring about premature development in many plant bulbs, tubers, rhizomes, dormant buds and other agamic reproductive formations, by changes in temperature or by short exposures to extreme temperatures. Although this practice has long been known to growers, the first experiments in this field of investigation were performed by Paulig (1905), Howard (1906), Molisch (1908—9) and by Iraklinow (1912). The literature on this subject has been reviewed by F. Weber (1922) in whose article many details are to be found. Two points deserve special attention in this connection, *viz.* that activation may be produced not only by low or high temperatures, but by many other agents, both physical and chemical, and that the success of the experiment greatly depends on the time of the year.

Vegis (1931, 1932) has recently investigated activation by heat of dormant buds of the *Hydrocharis*. He obtained activation by exposing the buds to 40°—55° for a period varying between 15 seconds and 6 hours, according to the temperature applied. The temperature coefficient of the process is high ($Q_{10} = 58$; $m = 22.5$).

It has often been noted that variations of temperature, which take place during development, stimulate growth. This was observed in certain insects by Abbe (1877, quoted from Bodine 1925) and by Bodine (1925), in molluscs by Bachrach and Cardot (1924). Kaestner (1895) assumed that such growth stimulation intervenes in the normal development of the chick. Likewise in plants Silberschmidt (1925) observed a reaction, which he calls "*Thermowachstumreaktion*", consisting in an acceleration of growth followed by a retardation, which takes place with any sudden change in temperature.

10. Theories of stimulative effect of temperature

The theoretical interpretation of the phenomena analyzed in this chapter obviously cannot be the same for those cases in wich stimulation is produced by extreme temperatures, as for

cases in which stimulation is caused by variations of biokinetic temperatures.

The most popular explanation of the first category of phenomena is that the stimulative action of extreme temperatures is caused by an accumulation of abnormal metabolic products, owing to partial asphyxiation (Boresch 1928, Gardner 1929, Hée 1929), or to a partial releasing of cellular enzymes (Iraklinow 1912, H. H. Meyer 1927). But it is very doubtful whether there is a sufficient accumulation of such supposed products in cases where the time of exposure is short (Vegis 1931, 1932). J. Loeb (1916) imagined that the protoplasmic surface-layer is destroyed by a sudden variation in temperature in a similar way as a glass plate bursts on sudden heating or cooling, whereas a slow temperature change of the same extent it not injurious. This idea however has not been generally accepted.

Stimulation by heat seems to be based upon the same process as that which underlies the injury by heat, as far as can be judged from the high temperature coefficient of thermic parthenogenesis and of heat activation of plant buds.

For the other category of phenomena, in which stimulation occurs by simply varying the temperature within the limits of the biokinetic scale, it seems that they must be considered as being identical, in their intimate mechanism, with the general process of excitation.

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